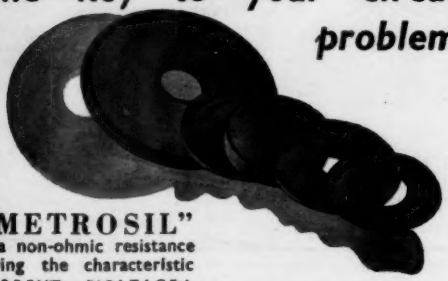
P 25

UNIVERSITY OF MICHIGAN
PERIODICAL READING ROOM
SEP 24 1956

VOL. 178, No. 4532 SATURDAY, SEPTEMBER 8, 1956 TWO SHILLINGS

PERIODICAL READING ROOM

The key to your circuit problems



"METROSIL"
is a non-ohmic resistance having the characteristic CURRENT ∝ (VOLTAGE)^4.

Some proved uses are in:
SURGE SUPPRESSION · LIGHTNING ARRESTERS · VOLTAGE REGULATION · FIELD CONTROL · SPARK SUPPRESSION · RADIO CIRCUIT PROTECTION

Send for leaflet NO. 220/1-1

METROPOLITAN-VICKERS ELECTRICAL Co. Ltd., Manchester, 17
Member of the A.E.I. group of Companies M/M 601

Tinsley

STANDARD CADMIUM CELLS

Single and double types in wooden cases or in metal cases as illustrated.

Reliable single or double miniature types mounted in plastic cases, E.M.F. 1.01859 abs. volts at 20°C. Temp. coeff.—40 μV/1°C. Low internal resistance.

Dimensions 3¼ in. × 1⅜ in. × 1 5/16 in.

Non-temperature coefficient cells for industrial use.

All types of unmounted standard cells can be supplied in quantity from stock.

H. Tinsley & Co. Ltd., Werndee Hall
London, S.E.25
Telephone: ADDiscombe 6046-7-8

Humidity Measurement & Control

Hair Element
Wet and Dry Bulb
Gregory Electrolytic

MERCURY-IN-GLASS
MERCURY-IN-STEEL
ELECTRICAL RESISTANCE

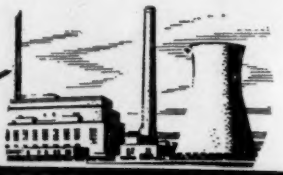
Methods

Send for List H/20: Post free

Negretti & Zambra, Ltd., 122 Regent St., W.1 **REGent 3406**

BRANCHES: *Birmingham · Cardiff · Glasgow · Leeds · Manchester · Nottingham*
AGENTS OVERSEAS: *Argentine · Australia · Belgium · Brazil · Canada · Czechoslovakia · Denmark Egypt · Eire · Finland · France · Greece · Holland · Iceland · India · Italy · New Zealand N. Ireland · Norway · Portugal · S. Africa · Spain · Sweden · Turkey*
Technical Service available in most countries.

The *simplest*, most *economical* way of distributing VACUUM *throughout* . . .



INDUSTRY

HOSPITALS

'Piped' vacuum is the modern, economical method of serving many users in ANY part of the factory or laboratory *at all times*. Simple and compact enough to be installed into any convenient cupboard, this multi-point unit provides an economical alternative to single pumping units wherever the individual demand is not so exacting as to require *highest* vacuum performance or complete freedom from the effects of multiple duties.

'SPEEDIVAC' AIR-BALLASTED ROTARY PUMPS are now fitted to these units to handle condensable vapours.

For better vacuum service . . .

EDWARDS HIGH VACUUM LTD

MANOR ROYAL · CRAWLEY · SUSSEX | CRAWLEY 1500 (10 LINES) | EDCOHIVAC CRAWLEY

CASELLA LONDON

THERMAL PRECIPITATOR

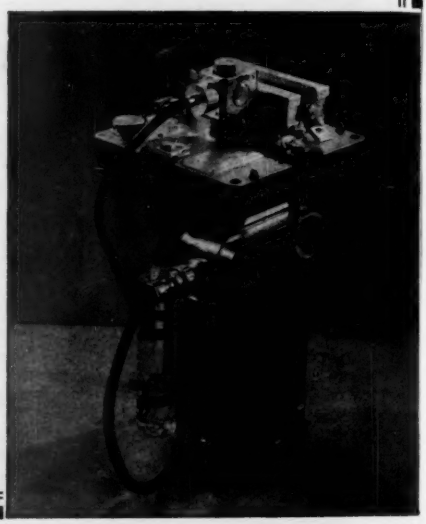
FOR SAMPLING AIRBORNE DUST CLOUDS

(IMPROVED MODEL)

This instrument enables organic or inorganic dust particles to be collected with an efficiency approaching 100 per cent for particles below 10 μ.

It has recently been redesigned and is now more compact, lighter, and more adaptable to awkward sampling positions.

7″×7″×14″ (17×17×35 cm.) 20 lb. (9 kg.)



C. F. CASELLA & Co. Ltd.

REGENT HOUSE, FITZROY SQUARE
LONDON, W.1

Telephone: ★EUSton 3944

Member Scientific Instrument Manufacturers' Association of Great Britain

NATURE

No. 4532 SATURDAY, SEPTEMBER 8, 1956 Vol. 178

CONTENTS

	Page
Health and Safety in Industry	505
Palæogeographical Reconstruction. By Prof. L. R. Moore	507
Progress in Semiconductors. By Dr. J. R. Tillman	508
Enzymological Recipes. By Dr. Edwin C. Webb	509
Motives in the Classroom. By Prof. E. A. Peel	509
Education in the Free World. By R. Brightman	510
Centenary of the University of Melbourne. By Prof. John S. Turner	512
The Antigenic Stimulus in Transplantation Immunity. By Dr. R. E. Billingham, Dr. L. Brent and Prof. P. B. Medawar, F.R.S.	514
Obituaries :	
Sir Harold Scott, K.C.M.G. By Dr. Charles Wilcocks, C.M.G.	520
Dr. E. Leonard Gill. By Prof. D. M. S. Watson, F.R.S.	520
Mr. John A. Marsh. By Prof. Alex. Haddow	520
News and Views	521
Applied Research and Development within the Industrial Group of the U.K. Atomic Energy Authority. By L. Rotherham and Dr. A. B. McIntosh	524
International Protection of Birds. By Miss Phyllis Barclay-Smith	527
International Scientific Radio Union	528
National Institute of Agricultural Botany, Cambridge : Report for 1956	529
Studies in Mutagenesis. By Rose M. Litman and Prof. Arthur B. Pardee ; Carroll A. Coughlin and Prof. Edward A. Adelberg	529
Letters to the Editors :	
The Sun's General Magnetic Field.—Dr. Horace W. Babcock	533
Determination of Changes in the Dimensions of the Earth from Palæogeographical Data.—L. Egyed	534
Magnetic Perturbation of the Lowest Triplet States of Aromatic Molecules by Dissolved Oxygen.—Dr. D. F. Evans	534
Pitch of Inharmonic Signals.—E. de Boer	535
Cleavage Fracture of Bismuth Single Crystals.—W. Vickers and Dr. G. B. Greenough	536
Detection of Iodine-containing Substances on Paper Chromatograms.—I. Dragúnová and Dr. P. Langer	537
Detection of Triterpenoid Glycosides on Paper Chromatograms.—I. Belic	538
Separation of Pyridine Mono-Carboxylic Acids on Paper Chromatograms.—Jiban K. Chakrabarti and Ajoy K. Guha	538
A Simple Method of *Rh* Determination.—Prof. F. Milgrom, S. Dubiski and G. Wozniczko	539
Carnosine Phosphate as Phosphate Donor in Muscular Contraction.—M. C. Goodall	539
Appearance of Serine upon Alkaline Incubation of Iodinated Polytyrosine.—Dr. Michael Sela and Sarah Sarid	540
Effect of L-Thyroxine on Carotene and Vitamin A Metabolism in the Cow and the Chick.—Dr. R. Chanda	541
Fluorescence of Adrenaline and Noradrenaline with Ethylene Diamine.—George P. Burn and E. O. Field	542
Spore-like Structures in the Tubercle Bacillus.—Dr. E. M. Brieger and Dr. Audrey M. Glauert	544
Effect of Electrolytes on the Rate of 'Supercontraction' of Wool Fibres.—W. G. Crewther and L. M. Dowling	544
Cytotaxonomy and the Migration of Primitive Peoples.—Dr. J. A. Rattenbury	545
Histamine of the Reptilian Stomach and Lung.—Prof. R. D. Wright and Dr. E. R. Trethewie	546
A Case of DDT Poisoning in Fish.—E. H. W. J. Burden	546
Occurrence of Paracolon Bacteria in a Tropical Marine Environment and their Classification.—A. Sreenivasan and R. Venkataraman	547
Availability to Dinitrofluorobenzene of some Amino Groups in Human Dentin and Ox Hide Collagen.—C. C. Solomons and Prof. J. T. Irving	548
Correlation between Deoxyribonucleic Acid Content and Volume of Individual Nuclei in Different Tissues of the Rat.—Prof. J. Fautrez	548
Human Fœtal Ventricular Outputs.—Dr. E. N. Keen	549
Delayed Implantation in the Grey Seal, *Halichoerus grypus* (Fab.).—K. Backhouse and Dr. H. R. Hewer	550
A Culture Technique for *Venturia* spp. and a Turbidimetric Method for the Estimation of Comparative Sporulation.—Dr. D. S. Kirkham	550
Intake of Streptomycin by Peach Leaves.—M. H. Dye	551
Effects of Temperature and Length of Day on the Sex Expression of Monoecious and Dioecious Angiosperms.—R. G. Thomas	552
Soil Transmission of Scottish Raspberry Leaf-Curl Disease.—Dr. B. D. Harrison	553

CHAPMAN & HALL

Just Published

MOLECULAR FLOW OF GASES

by

G. N. Patterson
(University of Toronto)

Size: 9¼″ × 6″ *227 pages* *Illustrated* 60*s. net*

This book is based on a series of lectures to advanced students in engineering and physics at the University of Toronto. No attempt has been made to give a complete treatment of either gas dynamics or the kinetic theory of gases. The essential purpose of the book is to assist the reader in making a transition in outlook from the continuum to the molecular viewpoint.

POLYESTERS
AND THEIR APPLICATIONS

by

Johan Bjorksten, Henry Tovey
Betty Harker, James Henning
(All of Bjorksten Research Labs. Inc., Madison, Wisconsin)

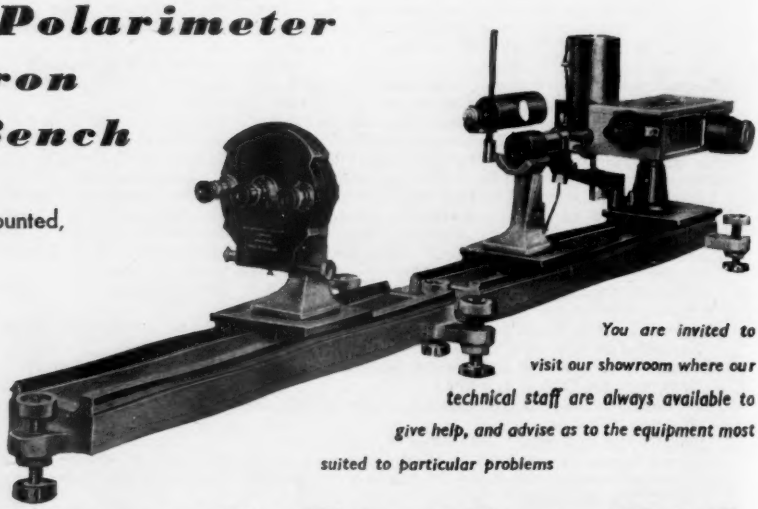
Size: 9″ × 6″ *626 pages* 80*s. net*

A comprehensive survey of the entire polyester field from raw materials to fabricated product. The text and annotated bibliography of over 3,300 references cover most phases of the production and use of polyesters, and as a source of valuable information on the literature of the subject is possibly unique.

37 ESSEX STREET, LONDON, W.C.2

PRECISION BS INSTRUMENTS

Model A Polarimeter on cast iron Optical Bench



Monochromator can be mounted, as illustrated, when desired.

Suitable for the insertion of auxiliary apparatus between polariser and analyser.

You are invited to visit our showroom where our technical staff are always available to give help, and advise as to the equipment most suited to particular problems

Bellingham & Stanley Ltd.

DEPT. N, 71 HORNSEY RISE, LONDON, N.19

PHONE: ARCHWAY 2270

HEALTH AND SAFETY IN INDUSTRY

NOT only Parliament but also public opinion in Britain generally was greatly shocked by the statement about the lack of precautions against fire in factories which was made in the House of Commons on May 18 by Mr. Robert Carr, Parliamentary Secretary to the Ministry of Labour and National Service. Mr. Carr's statement, lucid and admirable in itself, was made on a motion for the adjournment arising out of a fire in a Keighley woollen mill in February in which eight persons lost their lives and which was one of four occurring within a single month in Yorkshire woollen mills. Mr. Carr estimated that between 50 per cent and 70 per cent of the factories covered by the Factory Act of 1937 had no fire alarms; many of them were small and single-story buildings with extremely low fire risks, but nevertheless this is an excessive degree of non-compliance which Mr. Carr admitted could not be allowed to continue.

Most of his statement was devoted by Mr. Carr to an outline of the measures by which the Government proposes to secure greater compliance with the regulations. He said that he is against holding frequent public inquiries; but in the case of serious accidents involving loss of life and much damage, they might be very desirable from the point of view of public morale alone. As a first step, however, the Chief Inspector of Factories is writing to all factory occupiers covered by the regulations, reminding them of their obligation to instal fire alarms; and a rapid survey of the forty to fifty thousand premises affected is being made. A leaflet dealing with the installation of fire alarms and the types of fire alarm most suitable to different premises is also being circulated. Secondly, the Ministry is to speed up its examination of premises by instructing inspectors to make greater use of their existing powers, both in respect of notifying local authorities and also, where necessary, carrying out the examination themselves. Inspectors are being instructed to press employers to introduce fire-drill where suitable; and the decision whether or not to introduce regulations would be made in the light of the success attending such voluntary persuasion. The need for further special regulations to deal with abnormal fire risks is also being examined and it is proposed to intensify general advisory work on fire prevention, both by factory inspectors working in closer collaboration with the fire prevention officers and by publishing suitable literature on the subject.

In outlining this programme of positive action, however, Mr. Carr was careful to put the fire risk in its proper context. Injuries and fatalities caused by fire in industry in total are small, amounting to only one-quarter of one per cent of all the factory injuries reported to his Department. In all, about 180,000 people every year are injured sufficiently seriously to be reported to the Ministry, and some twenty million production days are lost every year as a result of factory accidents, or almost six times as many as were lost last year through industrial disputes. As Mr. Carr remarked, we cannot afford to continue the loss and suffering caused by these accidents.

Mr. Carr's statement received high praise when, some two months later, on July 16, the House of Commons debated the administration of the Factories Acts. Following Mr. F. Lee, who had opened the debate, Mr. Carr then emphasized that while the prime responsibility for health and safety in industry rests on industry itself—both employers and employees—the Government has a four-fold role. It must see that the factory law keeps in step with modern needs and developments; it must see that the minimum requirements laid down in the Acts and in the many codes of regulations made under them are observed; it must be able to advise industry as a whole on these matters; and it must undertake, and stimulate others to undertake, investigation, research and development. In this last connexion, Mr. Carr referred to the recent establishment of an Inter-departmental Committee on Industrial Safety Research, comprising representatives of government, research committees and the Ministry of Labour. Moreover, as a result of discussions with the Industrial Health Advisory Committee appointed in 1954, fresh investigations are proceeding, he said, under the auspices of the Medical Research Council and the Ministry of Labour, into health problems in various industries; for example, into dust problems in the foundry and cotton industries, the dangers arising from the use of toxic solvents and the widespread risk of dermatitis.

On the general situation, Mr. Carr said he believes it should be a matter for concern that the downward trend in the number of accidents reported each year under the Factories Acts up to 1952 had ceased, for during the past few years we have had about 160,000 accidents in factories and 25,000 accidents in other work places involving about 700 fatalities every year. There is a relative decline in accidents caused by power-driven machinery, and up to 66 per cent of all accidents in factories, as well as most accidents at other work places, such as building sites, are now caused in the handling of goods, by persons falling, stepping on or striking against objects, by falling objects or in the use of hand tools.

There are three important aspects of the whole question, with all of which the scientist and technologist are closely concerned, on which Mr. Carr also touched, particularly in speaking on July 16. The scientist and technologist obviously have often in the right of their special knowledge or experience an immediate contribution to make to industrial health and safety. That contribution is most apparent in the work of the Fire Research Organization and in that of the Industrial Health Advisory Committee and related bodies; but it will also be conspicuous in the 'good housekeeping' which Mr. Carr rightly said is an important factor in the prevention of accidents in industry.

Success here, however, does depend very largely on the existence of the right climate of opinion and, as Mr. Carr pointed out, employers and trade unions, jointly and severally, have a grave responsibility, both in education and in creating conditions conducive to the appreciation by everyone who works in industry of the need for safety. That, moreover, is no less true of the work of the factory inspectorate itself. Unsatisfactory standards of compliance with regulations under the Factories Acts and the failure of the inspectors to make greater use of the powers entrusted to them are due partly at least to the general climate of opinion. In the shock to public opinion given by loss of life, action is possible which cannot be sustained unless opinion has thus been brought to realize its necessity.

For legislation on safety matters to be effective, there must be the understanding which will ensure co-operation and support, not simply with industry but also on the part of local authorities and others. In the promotion of such understanding the scientist will often have an important part to play, and it is a responsibility which he should keep continually in mind. It may well be that such responsibility should be discharged increasingly through his professional associations, and this is a matter which should receive further careful consideration from such bodies.

The creation of favourable conditions for health and safety in the factory is, however, first and foremost a responsibility of management. Unless management has fully discharged that responsibility—and not merely complied with the legal obligations but established proper relations with the factory inspectors—the employee can contribute little or nothing, and his co-operation, even if it is secured, may be ineffective or frustrated. The position disclosed by Mr. Carr's statement on May 18 is a serious indictment of management. The evidence of neglect is disturbing; but still more alarming is the evidence of ignorance—not merely the ignorance of factory law, to which Mr. J. C. George and Mr. V. Collins referred, but still more the ignorance of scientific and technical matters.

If the first lesson to be learnt from the position disclosed as a result of the Keighley woollen mill fire is the importance of an alert and educated public opinion, within and without the factory, the second lesson is the vital importance of trained management. Even in the small factory, management to-day demands not merely skill in handling men but also an awareness of scientific and technical factors in many contexts which is not to be acquired without training in which science and technology must have some place. Quite as plainly as the advent of automation, considerations of health and safety in industry emphasize the need of education for management. Without some appropriate technical and scientific training, the manager of even a small works or factory cannot appreciate the factors which make for safety and for health, nor can he fully understand the warnings which a factory inspector may give.

To put the prime responsibility for industrial health and safety on management is not to deprecate the part of the factory inspectorate itself. While the inspectorate has emerged well from this debate, misgivings were freely expressed as to the ability of the Ministry to recruit inspectors of the quality required, and especially as to the numerical strength of the inspectorate. In his statement on May 18, Mr. Carr said that the Minister had just received the report of an inquiry by three senior officers into the staffing and organization of the factory inspectorate; and some recommendations about the number of staff have been accepted.

Excluding certain senior posts at headquarters, the present authorized strength of the general factory inspectorate in Great Britain is 322; ever since the end of the War, when it stood at 309, it has been difficult to recruit up to the full establishment, though the number of vacancies had been reduced to 14 in 1953 and the service is now virtually at full strength. Mr. Carr pointed out that the only practical way of organizing the work of the general inspectorate is on a geographical basis, and that if we are to depend for general inspection on technical qualifications, we shall need both more inspectors and an ever greater variety of different specialists. The work of the general inspectorate certainly requires a knowledge of technical matters; but it is in itself an executive job, and many arts graduates have made excellent inspectors. Moreover, increase of the technically qualified content of the inspectorate must be considered in the light of the urgent need for technical man-power throughout the whole economy of Britain, and Mr. Carr suggested that it is in staffing its special branches, and particularly the engineering and chemical branch, that the fullest use can be made of the relatively few with technical qualifications.

Mr. Carr said further that the load on the engineering and chemical branch is particularly heavy, and at the moment it is unable to give as much help as is desirable in dealing with inquiries from the districts or in dealing at first hand with all problems calling for specialized knowledge. Moreover, its work will be substantially increased by the decision to expedite production of several new codes and regulations and advisory publications. It is accordingly proposed to increase the strength of this branch from 18 to at least 44, thus permitting one engineering inspector and one chemical inspector to be stationed in each division. There will also be an increase in the general inspectorate, particularly in order to enable the inspectors to pay more attention to building operations and engineering constructional work.

While the increase announced by Mr. Carr did not satisfy those who had urged a considerable expansion in the inspectorate—of the order of 25–30 per cent—it was accompanied by a reasoned explanation of the basis on which, since January 1, 1955, visits of inspection have been planned. The Ministry is satisfied that the more flexible arrangements then introduced were on the right lines. There is more, however, in Dr. Barnett Stross's plea for expansion than Mr. Carr was disposed to concede. But whatever the increase in the inspectorate, whether in the numbers with technical qualifications, in women, as Mr. G. Nabarro urged, or in those with non-technical

qualifications, the effectiveness of its work largely depends on the support it receives not merely from within industry but also from public opinion generally.

It is in this connexion that salary scales can be important, and here again Dr. Barnett Stross's sustained argument deserves the widespread support of the professional bodies of scientists. It is not simply that, as was strongly urged in the debate, without reasonable salary scales the inspectorate will not attract men and women with the qualifications and character that are essential. If those scales are not comparable with those which men and women of such ability can obtain elsewhere, inspectors will not enjoy the standing in the community which will give the necessary weight to their recommendations and advice. This may well be increasingly true in a Welfare State in which attention is concentrated on material things.

There is a further factor to be considered here. The value of the inspectorate is derived largely from the experience it gains in observing and comparing the safety practices of the more progressive firms. A first-class inspector is able to put such experience at the disposal of new firms or of companies who are entering new and unfamiliar fields. For this to be done effectively demands first-class minds and men whose personality as well as knowledge command respect, and who are able to meet managers and directors on equal terms.

Such men—or women—cannot be obtained on the cheap, nor can we ignore the tendency already noted for their status and influence to be proportioned to the salary scales they enjoy. There are, as has already been indicated, many ways in which scientists and technologists can promote health and safety in industry—by co-operating in the task of education, by direct contributions to 'good housekeeping' and safety measures and practices and by their own professional competence as inspectors or managers. They should not neglect to see, however, that their professional associations lose no opportunity of promoting the formulation of safety codes and practices, or of strengthening the esteem and status to which the factory inspectorate in Great Britain is both richly entitled and on which the effective discharge of its responsibilities depends.

PALÆOGEOGRAPHICAL RECONSTRUCTION

Concealed Coalfields

A Palæogeographical Study of the Stratigraphy and Tectonics of Mid-England in Relation to Coal Reserves. By Prof. Leonard J. Wills. Pp. xiii + 208. (London and Glasgow : Blackie and Son, Ltd., 1956.) 40*s*. net.

THE essential core of geological science lies in the establishment of the nature and ordered succession of events which together constitute the history of the Earth's crust. It is therefore a noteworthy occasion when an attempt is made to fulfil this ultimate aim by the application of the total geological evidence available to a restricted number of events even over a limited area such as the Midlands and North of England. This setting of the pieces into the rational puzzle of living stratigraphy is the life-blood of geological investigation, and by this means the Earth becomes an organism with a physiology of its own. Not all the pieces can ever be present in this puzzle ; some have been removed by earlier operations, while other evidence is still deeply buried under the surface skin. It is here that diagnosis based upon related facts plays such an important part, and conjecture and reasoned hypothesis are essential tools. A complete recording of contemporaneous events and their succession represented in geographical pictures which will provide palæogeographical reconstructions of episodes in earth history is desirable. The essential pre-requisite is a sound chronology with short time-intervals, the pulse of the organism, such as can only be provided by effective zonal sub-division based on past life in the form of fossils. Even then, the success of reconstruction is dependent upon the nature and detail of the evidence available, and is furthermore subjective to the particular interest of the palæogeographer and to the fact that it is based upon a knowledge and interpretation of contemporary geological and geographical processes.

Despite these restrictions, careful analysis and diagnosis may unfold the distributions of sea or estuary, of lake or swamp, of mountain and valley, of desert and plain, and, in so doing, the vital physiology governing these occurrences may come to light. In such a manner an evaluation of the original distribution of the rocks is obtained and, in particular, the nature of the agents and processes both geographical and geological which have afterwards limited this distribution by separation into basins or blocks. When the rocks involved contain a national asset such as coal, the information is of prime importance.

With due humility, Prof. L. J. Wills has assembled the puzzle covering the succession of rocks which include the Coal Measures, Permian and Trias of formal stratigraphy. It would be difficult to find a more suitable succession of rocks for such a purpose. The chronology is well defined in the Coal Measures, there is wide diversity of palæogeographical change, and the factual evidence is as great as it could be due to mining and field mapping.

The first part of the book reviews the available stratigraphical evidence and contains the author's interpretation of this evidence and his methodology in its use. The introduction of new terms follows from the application of the evidence to palæogeographical studies, and sub-division of the rock succession from Coal Measures to Trias results. This is effected by the recognition of five 'Palstages' separated by six 'Palevents', a sub-division which does not necessarily follow that of established stratigraphical practice.

The 'Palevent' is a short-lived or sudden palæogeographical event of widespread occurrence and indicated by a marine horizon, a coal seam, or even terrestrial conditions involved in sudden changes of geography, and recorded by tectonic or new lithological features. These include the *subcrenatum* Marine Band, Cefn Coed–Aegir M.B., Brithdir–Tillery–Halesowen event (entry Tenuis zone), Second Malvernian movements, uplift of southern England which produced the Bunter Pebble Beds of the Midlands, and finally the transgression of the Rhaetic Sea. Doubtless other equally effective events could have

been used, but these would not necessarily amplify the cardinal points of the author's reconstruction.

The 'Palstage' represents a period of time during which conditions were fairly constant, or subject to consistent changes, or cycles of changes controlled by physical conditions. On this basis there is further sub-division into sub-belts which more nearly follow accepted stratigraphical sub-division.

In order to emphasize the economic aspect of the coal reserves and their relationship to the thick masses of rock which often overlie the main workable coals, the author has instituted a major sub-division within the succession comprising the Coal Measures–Permian–Trias successions. This is his Palevent 2, the widespread invasion of the Coal Measure scene by the Cefn Coed–Aegir marine transgression. Thus below this horizon are recognized the Sub-Aegir belt consisting of a Lower Coal Belt and the Main Coal Belt, the hiatus of coal production in the major British Coalfields, and that part of the sequence most likely to be involved in large-scale coal production. Above the Aegir horizon are Coal Measures (Supra-Aegir Belt), often typical, and which do in fact contain some workable coals; but the higher belts—for example, Haleskeele Belt, Desert Rocks Belt and Bunter–Keuper Belt—show great changes of conditions, particularly in the Midland and North of England coalfields. Thus all rocks above the Aegir Marine Band constitute a 'cover' to the Coal Belt. The lower cover is usually conformable to the Coal Belt and Carbo-Permian in age, while the higher New Red Cover is always unconformable. Thus all the major British Coalfields are, or were originally, "covered coalfields", while those with an unconformable New Red Cover are concealed in the normal sense. Such coalfields may be thus both covered and concealed both in entirety or in part.

The second and third parts of the book are concerned with the specific coalfields of the Midlands and Northern England, and the thicknesses and variability of the various palstages involved are given with the Aegir Marine Band used as a datum. This aspect both of cover and concealment is of important practical value in an assessment of the accessibility of the Main Coal Belt below. The entire cover is the sum of two very different sets of variable factors, and the structure of one part is not always coincident with that of the other. While the original thickness of the covers was controlled by contemporaneous movements, the residual thicknesses of both are mainly determined by erosion following uplifts. For example, the loss by erosion following faulting is greater than that following folding. Some idea of the complexity of relationship between the two covers and between these and the Coal Belt is gained from the thirty structural arrangements which are given for the concealed coalfields.

The complex Midland geological structures are proverbial, but summarily the author concludes that the controlling physiology is "the general principle of deep seated block jostling at depth". Herein lies the responsibility for repetition of movements along the same lines at different periods; these movements, operative in one sense or another from time to time, have led to geographical changes with resultant control over geological accumulation. At the same time the sharp folding and lateral but more usually vertical displacement along faults operative even to post-Triassic times were the resultants of these block movements. No book attempting palæogeographical reconstruction would be complete without adequate illustration; the Palstage maps are intriguing, while the structural maps, isopachyte maps, and plates showing the accessibility of the Main Coal Belt and of the highest worked seams, present the factual and hypothetical evidence in a manner which bears scrutiny.

This book with its original presentation makes a fundamental contribution to geological science, and its methodology constitutes an all too rare occasion in geological literature. There are criticisms which may be directed at the terminology and correlation, but these are outweighed by the value of the broader palæogeographical contribution. Similarly, diagnosis may often be a fickle jade, but it has to be made; better a reasoned hypothesis than even an inspired guess. Here is a pattern derived from the accumulated evidence of geological research in all its aspects, whether these be considered as 'pure' or 'applied'; and that pattern not only indicates the value of such scattered research, but also provokes an inspiring challenge to those concerned with the resources and possible future developments of an important national asset.

L. R. Moore

PROGRESS IN SEMICONDUCTORS

Progress in Semiconductors

Vol. 1. Edited by Dr. Alan F. Gibson, Prof. R. E. Burgess and Prof. P. Aigrain. Pp. vii+220. (London: Heywood and Co., Ltd., 1956.) 50*s*. net.

THE discovery of transistor action in 1948 set off many new studies of the physics of semiconductors; the search for satisfactory techniques of making transistors, phosphors and infra-red detectors led to many chemical and metallurgical studies. Application studies were also built up rapidly. So far, accounts of most of the later studies appear only in the original papers, the numbers of which are mounting rapidly and the collective reading of which is becoming an ever-increasingly formidable task. Relief has been afforded occasionally by some review articles, notably those appearing in the reports of two international conferences and in two issues of the *Proceedings of the Institute of Radio Engineers*. Something more regular is needed, however; integrating papers confined to, at most, one branch of the subject would be welcomed by newcomers to that branch and by workers in the other branches, and critical reviews would freshen the minds of all.

The publishers and editors of "Progress in Semiconductors", which is to appear annually, recognize the needs and have set out to fill them. This first volume has seven articles; physics predominates, not surprisingly in view of the editors' backgrounds and not unfairly in view of the proportion of research it still attracts in the subject. The first article, however, is more general; it summarizes the advances in silicon, from preparation and properties to applications. It is well balanced and factual, but leaves the reader to deduce the current problems. The next chapter directs attention to the important part played by germanium filaments in physical studies; it describes the preparation of filaments and the methods by which they have been used in measuring drift mobility, diffusion constant, life-time, surface recombination velocity, injection ratio at a point contact, and noise. The theory of the Seebeck effect in

semiconductors is explained in the next chapter. Thermoelectric effects with metals have for long provided thermometers; with semiconductors they promise other uses. The chapter is timely and should prove a useful reference for some years. Prof. G. F. J. Garlick contributes a chapter on the electrical properties of phosphors. Experiments can be more accurately defined now that single crystals of some phosphors can be grown, but results still need cautious interpretation. The next chapter explains the limitations of conventional junction transistors at radio frequencies and restates the merits of *p-n-i-p* and unipolar transistors. Written before the middle of 1955, it contains no mention of graded bases or of diffusion transistors. French authors provide the last two chapters, on the photo-magneto-electric (PME) effect and on field effects. The former effect has received much attention lately, and its finer points are better understood; it is being used to measure some basic properties of semiconductors and is well described here. Progress with field effects has been slow; the interpretation of results is hindered for lack of independent methods of measuring some surface properties.

The volume cannot escape some criticisms. The information content, per unit area, of some of the diagrams, particularly six at the beginning of the last chapter, is very low. The writing, in places, lacks the clarity and flow that one expects of good review articles—for example, those of "Advances in Electronics and Electron Physics", which admittedly has had several years in which to get into its stride. None the less, the new series has made a good start.

J. R. Tillman

ENZYMOLOGICAL RECIPES

Methods in Enzymology

Edited by Sidney P. Colowick and Nathan O. Kaplan. Vol. 2. Pp. xx+987. (New York: Academic Press, Inc.; London: Academic Books, Ltd., 1955.) 23.80 dollars.

THIS is the second of four volumes dealing with methods and techniques in enzymology, the first volume of which was published last year (see *Nature*, 177, 810; 1956). It completes the part of the series which is devoted to the preparation and assay of individual enzymes. The remaining two volumes are to cover preparation of substrates and special techniques used in the study of enzymes.

The 152 articles in this volume are arranged in five sections, namely: enzymes of protein metabolism; phosphate metabolism; nucleic acid metabolism; coenzyme and vitamin metabolism; and finally, respiratory enzymes. This arrangement of enzymes according to their functional relationship in the metabolic pattern tends to obscure important similarities between enzymes carrying out similar reactions; for example, the phosphokinases are scattered throughout the book, and indeed throughout Vol. 1 as well.

More than a hundred and thirty authors have contributed to the present volume, and the articles naturally vary considerably in style and quality, although there is a fairly standard system of headings, with sections on assay method, purification procedure, and properties for each enzyme. Some articles give a useful short review on the occurrence and metabolic importance of the enzyme being treated, together with several methods of preparation. In other cases the article is virtually a reprint from the original literature of a single paper on the preparation of the enzyme. The multiplicity of authors has led to a certain amount of duplication; for example, Elliott's method of preparation of glutamine synthetase from peas is quoted verbatim on p. 339 and again (under the heading of γ-glutamyltransferase, with which it is identical) on p. 264. In this case the reproduction of the original paper includes an inconsistency, most annoying to the worker who is attempting to use the procedure, which is probably a misprint in the original (3·7 l. is given as 0·05 vol. with respect to a volume which is more than 144 l.).

Only a part of the material in this book will be of any permanent value. When an enzyme has been obtained in a pure state in high yield, the method may become classical: but many of the preparations given are necessarily merely extracts of tissues of exceedingly low purity, which will soon become outdated as research on the enzyme in question proceeds. On the other hand, as a summary of present knowledge of enzyme purification, the two volumes are remarkably complete.

From such a large collection it is difficult to single out any for special mention, and in any event, such a mention may merely reflect the interests of the reviewer. However, as samples may be mentioned some excellent accounts of the proteolytic enzymes of digestion, of the more well-defined components of the blood-clotting system, of the bacterial system which cleaves aromatic rings, and of the enzymes which break down coenzyme I. The whole section on the enzymes of coenzyme metabolism is perhaps particularly useful, as many of these enzymes are of very recent discovery and it would be difficult for the newcomer to the field to track down the relevant papers. A certain amount of material in this section has not been published elsewhere.

There is no question that this is a work which should be in every biochemical library and will be of value to all who at some time or other, for research or teaching, have to prepare enzymes.

Edwin C. Webb

MOTIVES IN THE CLASSROOM

Educational Psychology in the Classroom

By Prof. Henry Clay Lindgren. Pp. xv+521. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1956.) 40*s*. net.

THE educational psychologist is increasingly turning his attention to classroom problems; but this does not mean that he has all to teach and nothing to learn. The situation in the classroom is, however, more complex than earlier generations of teachers assumed and calls for the kind of understanding that contemporary psychology can supply.

Prof. H. C. Lindgren's book is written in this tradition and in a helpful spirit for the teacher. He stresses the value of group methods, of active learning and of the partnership between teacher and taught. Authoritarian methods are condemned. We understand that unless a pupil likes school, little headway will be made, for in a way the teacher is leader of his class only in so far as he is made so by his pupils. Lindgren also draws on sociological studies such as "Elmtown's Youth" and insists that to know a

child's social class is very important to the teacher. He is well aware that, in its extreme form, this type of relationship may be peculiar to the American way of life; but the British reader will find much in these views worth giving a try.

Lindgren stresses also the relativity of human experience. Teachers often judge a pupil's actions in terms of their own values and experiences, failing to realize that a different set of values and perceptions may operate for the pupil. We need to look for less obvious motives; and to try to view things as the pupil sees them is to be commended.

In a book which emphasizes learning in and from the group in a relatively free and incidental way, learning theory is taken mainly from gestalt and field theory. In the last part there is material on assessing the results of learning, tests, guidance, children with special needs and the psychology of teaching. It is copiously illustrated with photographs and cartoons; but the British student may find them a little unusual and perhaps redundant. The text contains relevant non-technical examples, and there are suggestive exercises and full lists of references, particularly on the social and dynamic side.

The writer fuses the two themes of socialized group action and of a free learner enjoying equality of status with parent and teacher. On this matter of equality of status, I feel one must ask whether young children and even adolescents do not often expect adults to know more, to act as if they do, to impart their knowledge, to order the child's world a little and to allow him that amount of freedom he can manage. Freedom can be a burden as well as a boon. For all this, the book is a stimulating antidote for those who assume too readily the authoritarian role and hence are insensitive to the real forces in classroom life.

E. A. Peel

EDUCATION IN THE FREE WORLD

The Citadel of Learning

By James Bryant Conant. Pp. vii+79. (New Haven, Conn.: Yale University Press; London: Oxford University Press, 1956.) 16*s.* net.

THIS book is made up of three essays on the work of scholars and teachers and its relation not merely to the well-being of the United States but equally to the maintenance of freedom throughout the world, and the essays should command the attention and challenge the thought of many who will never be persuaded to read those scholarly volumes which have already appeared under the American Academic Freedom Project of Columbia University. It is no detraction from the excellence of the studies of Profs. R. Hofstadter, W. P. Metzzer and R. M. MacIver to suggest that in less than eighty pages Dr. J. B. Conant has given the gist of the story of the development of academic freedom in the United States and its meaning for the free society to-day. Indeed, although, especially in the second and third essays, he writes essentially in an American context, his wider vision gives his comments even greater relevance to the situation elsewhere than Prof. MacIver's penetrating comments and analysis, and British readers will be as grateful to Dr. Conant as those American readers to whom his volume is primarily addressed. It may be placed with those other notable contributions to Anglo-American understanding for which we are already indebted to a man who, as well as being a scientific worker and university administrator, lately served his country as United States High Commissioner and Ambassador to Western Germany.

The first of the three essays, which gives its title to the book, might be described as reflexions on the functions of a university provoked by a phrase freely translated from some words of Stalin. More particularly, Dr. Conant illumines the international function of a university—the advancement of learning—by examining what has happened among communities of scholars on the other side of the Iron Curtain, emphasizing that, from whatever angle art, literature, philosophy and science are viewed, they have been the outcome of both conflict and co-operation, a struggle among beliefs and a sharing of beliefs. Such creative activities are not confined to a university, but these are the only terms on which those within the university have lived and worked effectively for the advancement of learning in the past, and they are the only terms which will ensure the safety and vitality of the university as a citadel of learning in the future. Freedom and tolerance, Dr. Conant argues, go hand in hand in matters of the spirit; controversy is essential to a healthy condition in such a citadel.

In his second essay, entitled "An Old Tradition in a New World", Dr. Conant summarizes in masterly manner the re-shaping, within a century, of European ideas about schools and universities into a characteristic American tradition expressing the two ideals of equality of opportunity for all youth and equality of respect for all honest citizens. His summary, moreover, is critical, for though Dr. Conant believes these two ideals or doctrines are as significant for the future as for the past, he is not satisfied that American education has in all respects even started to outline the problems which the new position of the United States in the world has forced upon it. Some of these problems are indicated; by and large the same problems confront education in Britain to-day, so that this essay not only contributes to the understanding of American institutions and problems but should also stimulate thought among British readers about their own problems and the adaptation of their own institutions to the needs of the world in which we live.

When, in his last essay, Dr. Conant focuses his attention on some basic problems of American education, he is no less stimulating and provocative of fresh thinking to readers of other nationalities in considering problems of professional and technological education, the place of research in a university and the relations between teaching and research. There is a place for the heretic and the spirit of free inquiry in the education of professional men and women. "If the free world is to preserve that spirit which has made possible the growth of Western culture, the future leaders must as young men be impregnated with this spirit. They should early come to learn the significance of dissent and be exposed to an intellectual atmosphere where vital differences of opinion are not merely tolerated but encouraged." Dr. Conant's shrewd comments and criticisms point to dangers which are encountered in Britain no less than in the United States, and his wise counsel bears as closely on the expansion of British university institutions as it does on the re-shaping of American institutions to meet growing demands.

R. Brightman

Control of Nuclear Reactors and Power Plants

By M. A. Schultz. (McGraw-Hill Series in Nuclear Engineering.) Pp. viii+313. (London: McGraw-Hill Publishing Company, Ltd., 1955.) 52*s*. 6*d*.

THE author sets out to analyse the characteristics of nuclear power plants, primarily using servo techniques. As an example of the method, skilfully applied to a set of problems, the work is of value; although perhaps more to those whose interest is in the method rather than the problems. This opinion is advanced for two reasons. First, the servo method gives qualitative information on stability, rather than quantitative data on the course of transients. The latter problems, in my experience, predominate in the field of reactor control. Particularly in the base-load power reactors now being designed in Britain, there is little difficulty with stability against oscillatory tendencies, simply due to the wide spacing of the time-constants of the effects which comprise the control sequence. Certain problems might conceivably arise from distributed effects, but these would not be amenable to servo methods of solution. Second, the design of a control system for a nuclear reactor is a project which justifies considerable, one might say unlimited, attention and effort. If the particular equations cannot be solved by conventional methods, numerical solutions using digital computors, or graphical solutions using analogue computors, could be obtained. The speedy methods of frequency diagrams, admirably adapted to the repetition design of similar systems, seem too cursory in the present context.

The curves of Figs. 3.1 and 3.2 appear to be reproduced on too small a scale to be useful, and, so far as they can be read, do not satisfy the obvious condition $\Sigma A_j = 1$.

On p. 122, eqn. 5.3 should read $v = A\omega \cos \omega t$, and on the same page, the statement "rod mass $= m$, in poundals" should presumably read "in slugs".

Referring to Fig. 10.7, the point of inflexion at 0·1 sec. suggests that a period of artificial acceleration is followed by deceleration, followed by acceleration again under gravity. This does not correspond with the explanation in the text.

J. H. BOWEN

High Vacuum Technique

Theory, Practice, Industrial Applications and Properties of Materials. By J. Yarwood. Third edition, revised. Pp. vii+208. (London: Chapman and Hall, Ltd., 1955.) 25*s*. net.

SINCE the end of the Second World War, when the second edition of J. Yarwood's well-known and useful handbook of vacuum practice was first published, both the demand for, and the supply of, vacuum apparatus have increased manifold. In addition, several innovations have been introduced: ultra-high vacua of 10^{-10} mm. of mercury and less have been attained and measured, and many new applications of vacuum procedures have been made. Nevertheless, fundamentally, vacuum practice remains the same, and in the third edition of his monograph Yarwood resists quite rightly the temptation to omit the standard basic material in favour of more attractive modern applications of vacuum techniques to industrial processes.

The text is illustrated by a large number of line-drawings which are remarkably clear, well drawn and beautifully reproduced. There are an extensive bibliography and numerous references to scientific articles and manufacturers' catalogues. Many useful data are contained in the several tables which are scattered throughout the text. One wonders, however, why the author did not adopt some standard accepted form for the abbreviations of the titles of the periodicals to which reference is made, and in addition why some authors' names are quoted in full and others not. But these are trivial blemishes, and the volume still remains one of the best elementary text-books on the subject of vacuum technique to which to introduce students of science, and for industrial and other research workers to have close at hand for rapid reference.

Methods of Mathematical Physics

By Sir Harold Jeffreys and Bertha Swirles (Lady Jeffreys). Third edition. Pp. x+714. (Cambridge: At the University Press, 1956.) 84*s*. net.

THIS important work first appeared in 1946 (see *Nature*, **160**, 139; 1947); four years later a second edition, considerably revised, was issued. A third edition is now called for, and the authors have made further improvements, though on a much smaller scale. "Some theorems are stated more explicitly, a few proofs are added, and some are shortened"; others are generalized or given in more detail. After ten years, a book so widely used must now approach complete freedom from errors and misprints, thanks to the aid of many readers. The index combines subject and author references; the latter might appropriately include mention of acknowledgment in the prefaces.

Intuitionism

An Introduction. By Prof. A. Heyting. (Studies in Logic and the Foundations of Mathematics.) Pp. viii+132. (Amsterdam: North-Holland Publishing Company, 1956.) 13.50 guilders; 27*s*.

TO the intuitionist, mathematical existence is synonymous with construction; to inquire whether a number which cannot be constructed nevertheless exists is to go outside the domain of mathematics, and enter that of metaphysics. The programme is positive, but may also claim the negative virtue of avoiding some of the paradoxes which beset the logical foundations of classical mathematics, so the classical mathematician may well wish to know how much of his domain must be sacrificed to purchase this freedom, and to see, for example, how a theory of measure and integration can be built up in a domain in which a bounded monotonic sequence need not tend to a limit. Under Brouwer's primacy, much work has been done in The Netherlands, and Prof. A. Heyting, of Amsterdam, has had the happy idea of producing a missionary tract. In the first chapter, a dialogue between an intuitionist, a classical mathematician, a formalist and others sets the stage and the intuitionist offers to produce his wares for inspection. Thus chapters on algebra, integration, logic, are specific examples of intuitionistic mathematics at work, cutting a cautious path through the tangle of difficulties, with a good deal of useful comment. Reading the book still requires the utmost concentration, but granted this, the fascination of the topic begins to emerge and it may well be that some young mathematicians will be encouraged by it to meet Prof. Heyting's call for more workers in this field. Those who feel themselves to be too old to learn new tricks will nevertheless be grateful to the author for this successful attempt to explain the intuitionist point of view and programme.

T. A. A. BROADBENT

CENTENARY OF THE UNIVERSITY OF MELBOURNE

By Prof. JOHN S. TURNER
Department of Botany, Melbourne

THE University of Melbourne held its centenary celebrations during the week beginning August 13. Delegates from a hundred universities presented addresses. The oration was given by Sir Ian Clunies Ross, and fifteen honorary degrees were conferred in the new Wilson Hall. There were Vice-Regal and Government receptions, and the Council of the University gave a banquet in the Hall at the conclusion of the ceremony.

To-day the metropolitan area of Melbourne sprawls over some 200 square miles and houses 1½ million people, more than 60 per cent of the population of the State of Victoria. In its University there are about eight thousand staff and students. In 1840 less than half this number of people were living in the raw pioneer township on the banks of the Yarra. Twelve years later gold was found, and the young Colony began its first hectic burst of growth. The opportunities were then even greater than now. Hugh Childers, later to be British Chancellor of the Exchequer under Gladstone, and just down from Cambridge, became Auditor-General in 1852, and a year later he put through an Act to found the University of Melbourne. This was four years before the first election to the new Parliament. In 1854 Mr. Justice Redmond Barry became the first chancellor. The obvious site for the University, the Domain, now the centre of Melbourne's great system of parks, was then covered with the tents of migrants, and 100 acres were allotted to the University two miles out of town in almost virgin bush. Here was built the first wing of a small stone Gothic quadrangle, which served the University as its home for another twenty years.

The University made slow progress in a community of pioneers and gold-miners. During the next fifteen years it granted only a hundred degrees. Nevertheless, it was wisely served by its first administrators and teachers. In 1853 there were only four universities in Britain, and Melbourne, therefore, had an unrivalled opportunity of attracting first-rate men to its chairs. The first four professors who lived and worked in the little stone quadrangle were exceptional men. Two of them were from Ireland: Sir Frederick McCoy, F.R.S., the first professor of natural science and the founder of Melbourne's National Museum, and W. E. Hearn, the great professor of law and history. M. H. Irving (classics) was from Oxford, and W. P. Wilson (mathematics) from Cambridge. They were followed by G. B. Halford in 1863, the holder of the first chair of the new Medical Faculty.

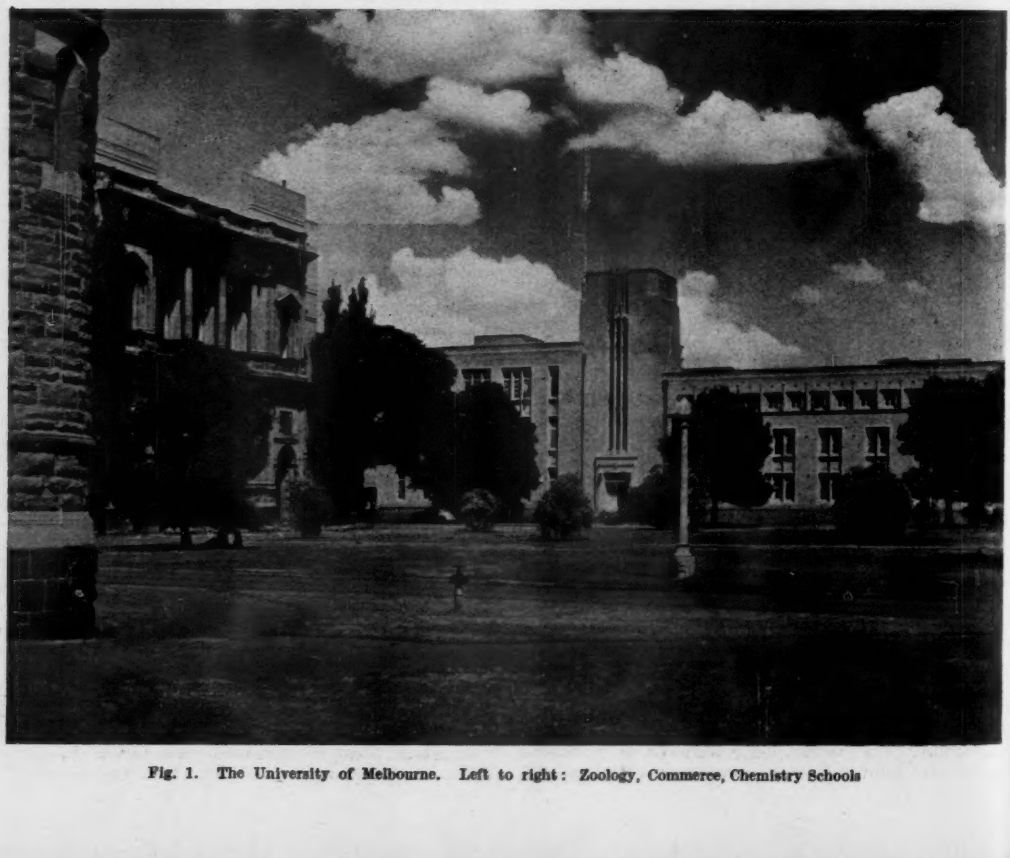


Fig. 1. The University of Melbourne. Left to right: Zoology, Commerce, Chemistry Schools

[*Photo : Visual Aids, University of Melbourne*

Fig. 2. Wilson Hall, University of Melbourne. The Premier of Victoria, the Hon. H. E. Bolte, addressing the meeting at the opening of Wilson Hall by the Governor, Sir Dallas Brooks

The University began its first move forward in the 'eighties. By that time most of the professorial houses were ready in the grounds—great red-brick Victorian mansions, now largely used for housing the overflowing departments. Five new professors, four of them Melbourne men, were appointed in 1882, and they were soon joined by four newcomers from Britain, who set a standard of scholarship and learning which Melbourne has consistently retained. They were T. G. Tucker (whom Cardinal Newman called the first Greek scholar of the day), David Orme Masson, chemist, Baldwin Spencer, the biologist who pioneered anthropological work on the aborigines, and Thomas Lyle, the first of a number of distinguished physicists. All three scientists became members of the Royal Society of London, and were later knighted, and their names are still remembered with affection and gratitude. The reputation they built up for the infant University attracted others, and out of a long list it is perhaps fitting to mention Sir William Harrison Moore (law), Sir Ernest Scott (history), E. J. Nanson and J. H. Michel, F.R.S. (mathematics), T. H. Laby, F.R.S. (physics), Sir Charles Martin, F.R.S. (physiology), J. W. Gregory, F.R.S., and E. W. Skeats (geology), W. C. and W. N. Kernott and H. Payne (engineering), W. R. Boyce Gibson (philosophy), G. W. L. Marshall-Hall (music), Sir Harry Brookes Allan (pathology), A. J. Ewart, F.R.S. (botany), W. E. Agar, F.R.S. (zoology), R. J. A. Berry and F. Wood-Jones, F.R.S. (anatomy) and L. F. Giblin (economics).

The University has been fortunate also in its administrators—it has had great legal chancellors like Sir Redmond Barry, Sir John Madden and Sir Charles Lowe, and an able succession of registrars. It is governed largely on British and Scottish lines, and although the supreme control is in the hands of a lay Council, a great deal of the government of the University is left to the Professorial Board and the Vice-Chancellor. The University made its greatest step forward into the modern period when it appointed Mr. (now Sir) Raymond Priestley as its first full-time vice-chancellor in 1936. Although he stayed only three years, his drive and energy saw the completion of the fine new Students' Union and the new Chemistry School. An inspiration to the staff, he was also instrumental in bringing town and gown together into a collaboration which resulted, under the present vice-chancellor, in the centenary appeal which raised more than half a million pounds. Priestley was followed by Sir John Medley, from Winchester and New College, Oxford. A man with wide contacts in the educational and business worlds of Australia, he brought to the University something of the grace of another period which is badly needed in an age of technocracy. He had to cope with the extremely difficult post-war period, when student numbers rose to alarming proportions. Under his

rule, and that of his successor, Prof. G. W. Paton, an unusually friendly spirit of co-operation arose between the members of the different faculties. The University has remained well balanced in the humanities and science, and has built up exceptionally strong medical and technological faculties over the past seventy years.

As may be expected in a metropolitan university, only a small proportion of the undergraduates live in the five colleges. Four of these are church foundations, and they have played an important part in the University, largely owing to the ability of their heads, of whom the Rev. E. H. Sugden, Sir John McFarland, Dr. Alexander Leeper, Sir John Behan, Dr. D. K. Picken and Father Murphy are the best known.

The University still occupies its original site, and here are gathered twelve faculties, a staff of 427 full-time academics with 812 part-time teachers, and a student body of 7,500. The growth of the University has been largely unplanned, and the architectural styles vary from the original Gothic of the quadrangle to utilitarian red brick, Glaswegian academic, Scottish baronial, Renaissance and the plain Army hut, the fruit of two world wars. Lawns and some trees still survive from McCoy's original ambitious plantings, and there are now signs of considerable improvement. The Union, Chemistry School, Geology wing, the new Arts block and, more recently, the Beaurepaire Physical Education Centre and the magnificent new Wilson Hall (which replaces an early benefactor's Gothic Hall) are a sign of a new dignity to come. With a massive immigration and considerable inflation, the University is only slowly finding it possible to erect new departments; but the long-awaited Library is about to rise, and with it new engineering buildings and the first floors of a new Biochemistry School, the gift of Sir Russell Grimwade.

Since the Second World War the University has taken an increasing interest in the Near East, and there are some three hundred Asian undergraduates reading for degrees at present. A new college, International House, is nearing completion, and this will accommodate equal numbers of Australian and overseas students. Several senior members of staff have travelled recently in Asian countries to advise on educational matters, and a new Department of Indonesian Studies has been set up.

The University has played a great part in the training of leading public and professional men in Australia and elsewhere. This is not the place to assess its achievements in scholarship; but it must be remembered that until recently little attempt was made to encourage indigenous research schools above the M.Sc. level. A characteristic of the young Australian is his urgent desire to see the world, and especially Britain, and this has led to a constant outward stream of graduates, mainly to Oxford, Cambridge and London. While we must be grateful to these universities for the training they have given to these men and women, it must not be forgotten that they, in their turn, have undoubtedly added to the prestige of the research schools in Britain. The Ph.D. degree was instituted in Melbourne in 1946, and now most departments are building senior research groups. This is a matter of some difficulty because of the increasing demand for our students by industry and the Government services. The future of the University is at present in the balance. It is a hundred years old, but active and vital, still growing rapidly and bursting at the seams. In spite of large annual financial grants by State and Commonwealth Governments and some splendid endowments, the University will not be able to cope with the demands which will shortly be made upon it. The State high schools are accepting six thousand new enrolments and the Education Department is building ten new high schools per annum. It must often be difficult to decide on the proper date for the foundation of a new university; but no such problem exists in Melbourne to-day. The State needs another university and it needs it urgently. The public men in Victoria are not without vision, and ambitious schemes are already in operation which will ensure the provision of power and water for a greatly increased population. At this time of centenary we confidently look to another Childers to found a second university, so that the old one may mature to its full greatness.

THE ANTIGENIC STIMULUS IN TRANSPLANTATION IMMUNITY

By Dr. R. E. BILLINGHAM*, Dr. L. BRENT and Prof. P. B. MEDAWAR, F.R.S.
University College, London

1. Introduction

'TRANSPLANTATION IMMUNITY' may be defined as the state of acquired resistance which leads to the destruction of homografts, that is, of grafts transplanted between two different members of the same species. The chemical nature of the substances that provoke it has so far defied analysis. It is widely believed, for want of clear evidence to the contrary, that only *living* cells can elicit transplantation immunity; and this belief appears to be upheld by the fact that an otherwise highly active suspension of living spleen cells or leucocytes loses its antigenic power after treatment in any of the following ways: heating to 48·5° C. for 20 min.; drying from the frozen state; or freezing and thawing three or four times. It will be shown here that the power of cells to elicit transplantation immunity depends neither upon their being alive nor upon their structural integrity in any anatomical sense; and that the antigens responsible for skin transplantation immunity are certainly nuclear substances, and probably desoxyribonucleoproteins.

We have worked with mice of two strains (domestic sublines of strains *A* and *CBA*) acting as donors or recipients at will. These strains differ from each other by upwards of 10–15 genetically distinguishable antigens which affect the survival of skin homografts[1]. The median survival times of skin homografts transplanted from *A* to *CBA* mice or vice versa are as follows: $A \rightarrow CBA$, $11{\cdot}0 \pm 0{\cdot}3$ days; $CBA \rightarrow A$, $10{\cdot}2 \pm 0{\cdot}3$ days[2]. These figures refer to mice receiving homografts for the first time. A second homograft, transplanted after the rejection of a first, is destroyed within six days[3]: healing is weak,

* Research Fellow of the British Empire Cancer Campaign.

vascular breakdown is virtually instantaneous, and the epithelium of the graft survives, for so long as it survives at all, in an entirely indolent state. This accelerated breakdown of 'second stage' homografts has been made the basis of a simple direct test for the existence of a state of immunity or sensitivity. First, mice are injected with the preparations that are to be examined for their antigenic power; then, three to five days later, they are grafted with skin of the same genetic origin as the substances under test. The homografts are inspected six days after their transplantation. Complete or advanced breakdown by the sixth day reveals the existence of a powerful state of immunity, for a normal homograft of six days standing is soundly healed, fully surviving, well vascularized, and exuberantly hyperplastic.

The problem, then, has been to find out the source and nature of the substances which, injected into mice beforehand, cause skin homografts to break down about twice as rapidly as would otherwise have been the case.

An analysis making use of the principle of immunological tolerance had already shown that the antigens responsible for skin transplantation immunity are fully represented in all the living tissues of a single individual[4]. It was therefore not necessary to seek in skin itself the antigens which incite immunity against skin homografts. Most of our work has been done with splenic (wholly mesenchymal) and kidney (almost wholly epithelial) cells. Moreover, advantage has at all times been taken of the fact that the members of a single highly inbred strain of mice are for most practical purposes antigenically equivalent[5], so that (for example) the spleens or kidneys of a dozen *A*-line mice may be used as a source of antigens, and skin homografts from still other *A*-line mice may be used for testing for any state of immunity which those antigens might have provoked. The technique of skin grafting and of scoring grafts for their degree of survival has been described elsewhere[3].

2. Immunity caused by Disintegrated Cells

Spleen cells totally disrupted by ultrasonic irradiation in any of the following media were found to be powerfully antigenic: (*a*) buffered normal saline (0·15*M* sodium chloride, 0·001*M* phosphate buffer, *p*H 7·4); (*b*) normal citrate saline (0·135*M* sodium chloride, 0·015*M* trisodium citrate adjusted with citric acid to *p*H 7·4); (*c*) isotonic sucrose, 0·29*M*; and (*d*) water.

Cell suspensions were made by dividing clean, freshly removed *A* or *CBA* spleens (mean weights 112 mgm. and 78 mgm. respectively) into three or four parts and expressing the pulp through a fine-meshed stainless steel sieve by gentle but firm pressure with a flat-ended glass pestle. Connective tissue was retained by the sieve, and the larger cell clumps in the expressed pulp were broken up by sucking them in and out of a pipette. The mean cell yield was 221 m (m = 10^6) per 100 mgm. wet weight of spleen[6], a figure which includes naked nuclei as well as whole cells, because even quite gentle manipulation with a pipette will cause upwards of 20 per cent of spleen cells to be stripped of cytoplasm. In media lacking calcium ions this can be a minor nuisance, because with anything but the most gentle handling some of the cells are trapped and lost in fibrous clots caused by nuclear disintegration.

Cells to be disintegrated in media *a* or *b* were expressed into those media, washed by two or three centrifugations at low speeds, and resuspended to a final concentration of 100–200 m./ml. Cells ultimately to be resuspended and disintegrated in isotonic sucrose (*c*) were expressed into and washed in isotonic sucrose – calcium chloride (see below). The handling of cells to be extracted in distilled water is described under sub-heading 6.

For disintegration, aliquot volumes of 2·5–3·0 ml. were loaded into siliconed glass tubes (internal bore 10 mm., thickness 1 mm.) and held obliquely in the jet arising from a 340-kc./s. crystal transducer housed in transformer oil. Although longer and stronger exposures were admissible, it proved sufficient to expose each tube for 30–40 sec. to a nominal acoustic power output of 7·5 W./cm.^{2} (applied voltage 0·75 kV.)[7]. Latterly we have used a 50-W. 20-kc./s. transducer of the magnetostrictive type: the flat end of a parallel and vertically held half wave-length stub, coupled to the transducer, was applied to the surface of a 6–7 ml. volume of the suspension under treatment. 45–60 sec. exposure sufficed, provided that the exposure vessel was not more than 2–3 mm. wider than the stub. It will be noticed that these exposures fall very far short of what is necessary to disrupt bacterial cells[8].

Cells disintegrated in normal saline or normal citrate saline were broken into fine fibres of nuclear origin, interspersed with cytoplasmic matter, red-cell debris, etc. Antigenic activity was confined to the heavy insoluble fibrous fraction, which could be cleared of cytoplasmic and soluble matter by two or three sedimentations on the centrifuge at low speeds. In isotonic sucrose the cells were broken into very small round particles, the smaller of which were not sedimentable by ordinary centrifugal forces. The antigenic activity of such a preparation was only just perceptibly reduced by centrifugation at 2,100 *g* for 20 min., that is, by exposure to more than twice the centrifugal force which, in less than half the time, would have sufficed to clear all whole cells or intact nuclei from a medium of this specific gravity; nor could the antigenic matter contained within a column 2 cm. deep be wholly sedimented by centrifugation at 11,000 *g* for 10 min.—an observation singly sufficient to dispel any idea that the antigenic activity of these 'particulate' preparations was due to contamination by whole nuclei or undisrupted cells. The specially important properties of the solutions formed by disintegrating cells in water are discussed under sub-heading 6.

Preparations made in media *a* to *d* were of about equal potency; the dosage (in cell equivalents) required to elicit violent immunity, such that a skin homograft transplanted three to five days after their injection would be destroyed within six days, was about 150–200 m per mouse. These experiments showed that transplantation immunity could be provoked by totally disrupted cells, but they gave no direct anatomical evidence of whereabouts in the cells the antigenic substances were to be found.

3. Location of the Antigens: Nucleus or Cytoplasm

Preparation of nuclei from spleen or thymus cells. Nuclei may be separated from spleen or thymus cells or leucocytes by fractional centrifugation after exposure to ultrasound in media containing calcium ions, namely: Ringer phosphate (containing 0·0028*M* calcium chloride, 0·0025*M* magnesium chloride, 0·001*M* phosphate buffer *p*H 7·4) or isotonic sucrose –

calcium chloride (0·0018*M* calcium chloride[9]). Cell suspensions prepared by the use of a sieve were washed two or three times by spinning at low speeds and then exposed to ultrasound of the intensity and duration described above. The nuclei were then separated by the use of barely sufficient centrifugal forces: two or three successive spins for 8 min. each at forces ranging from 200 to 700 *g* (for Ringer phosphate) or from 300 to 1,000 *g* (for sucrose–calcium chloride) down a fluid column height of 8–10 cm. would clear 2,000–4,000 m nuclei of visible cytoplasmic matter. The final preparation consisted of clean nuclei mixed with fibres of approximately the same specific gravity which could be shown to be artefacts of nuclear origin. Because of losses during exposure to ultrasound and the washes that preceded and followed it, only some 40 per cent of the original cells could be recovered as whole nuclei (mean yield 88 m/100 mgm. spleen). This yield could be improved by cutting down the time or intensity of exposure, but only at the proportionately increasing risk of allowing some nuclei to retain a cytoplasmic sheath. Unduly prolonged exposures reduced the yield of nuclei and increased the proportion of nuclear fibres.

Nuclei from kidney cells. Nuclei may be harvested from kidney cells by the judicious use of a high-speed blender. Clean decapsulated kidneys were cut into small pieces and washed in normal citrate saline. The pieces were then immersed in Ringer phosphate and exposed in a 100-ml. 'Vortex' flask to the action of an M.S.E. blender for 3–3½ min. at approximately 10,000 r.p.m. At this stage the preparation contained free nuclei, red cells, large quantities of cytoplasmic matter, and a multitude of short lengths of kidney tubules, but *isolated* living cells were extremely rare. The tubule segments were removed by repeated filtrations (if necessary, as many as fifteen) through a 3-cm. deep column of loosely packed, well-rinsed glass wool; to avoid too great a loss of nuclei, not more than two filters were used. The nuclei in the filtrates were concentrated, and freed so far as possible from cytoplasmic matter, by four successive fractional centrifugations at low speeds. The final preparations contained nuclei together with about one-tenth their number of red cells, and they were appreciably contaminated by larger cytoplasmic particles and any agglutinates of smaller particles that may have settled with them. The presence of nuclear fibres, usually coinciding with an absence of red cells, was a sign that blending had been unduly fierce or prolonged. The nuclear yield was variable, but more than half our preparations fell within a modal class of 20–30 m per kidney. A greater yield is not to be expected, for the essence of this technique is that kidney tubules should *not* be cut up into cell-sized fragments. The nuclei are presumably extracted from the tubules by cavitational forces.

Test for the presence of intact cells. One ml. of a suspension containing 10–50 m nuclei in Ringer phosphate was exposed to the action of 0·01–0·10 mgm. trypsin for about 20 min. at room temperature. Naked nuclei are extraordinarily vulnerable to the action of trypsin; intact cells may therefore be spun down and looked for microscopically. Twelve and nine such tests on separate preparations of spleen and kidney nuclei revealed the presence of intact cells in three specimens and in one specimen respectively, but the degree of contamination could not, at the extreme, have exceeded 0·1 per cent. As a significant source of error, contamination of nuclei by living cells may be disregarded.

Preparation of cytoplasmic matter. It did not prove feasible to prepare a concentrated suspension of spleen cell cytoplasm uncontaminated by fine fibres of nuclear origin; but cytoplasmic particles have been prepared from kidney and liver by orthodox methods using isotonic sucrose[10] or normal saline as diluents. Having cleared the preparations of nuclei or nuclear matter, suspensions consisting predominantly of large or of small cytoplasmic particles were prepared by the use of moderate (5–8,000 *g*) or of high (25–40,000 *g*) centrifugal forces.

Location of antigenic substances. The substances which elicit skin transplantation immunity are exclusively confined to the nuclei of cells: preparations of kidney or liver cytoplasm (whether of larger or smaller particles, in high or in low dosages) were totally inactive. Indeed, in so far as they had any effect at all in the concentrations at which they were used, it was to reduce the homograft reaction below its normal level of intensity. Very highly concentrated suspensions of the scanty cytoplasm of spleen cells have occasionally been found to exercise the feeblest perceptible antigenic action, presumably caused by the fine nuclear fibres with which such preparations have invariably been contaminated.

4. Properties of Nuclei

A nuclear dosage of 100 m per mouse is more than sufficient to cause the total breakdown of a test homograft within six days of its transplantation; 25 m/mouse is an appreciably active dose. Under the same conditions of administration, 100 m nuclei are equivalent to about 5 m dissociated living cells. It is convenient to inject nuclei intraperitoneally at a concentration of 100 m/ml.; the intravenous route is at least as effective, but the subcutaneous route is much less satisfactory.

The injection of mice with nuclei or nuclear derivatives originating from other members of their own strain had no discernible effect upon their immunological responses.

Nuclei are unstable, and although we have not examined the consequences of doing otherwise, we have always prepared and kept nuclei in chilled media. Unlike living cells, nuclei could not be stored overnight in a balanced saline medium at 3°–5° C. without losing all but a small fraction of their antigenic power. The antigenic activity of nuclei or of totally disintegrated cells was reduced beyond a perceptible level of intensity by any of the following treatments: heating to 48·5° C. for 20 min., drying from the frozen state, or freezing and thawing three times. (Nuclei suspended in sucrose–calcium chloride were weakly protected against the damaging effects of these treatments.) With this evidence of instability it is not surprising that, in the past, antigenic activity should have been regarded as the prerogative of living cells. None of the treatments just described, except freezing and thawing in a salty medium, caused gross anatomical damage to the nuclei; the mere appearance of nuclei under the microscope is a remarkably poor guide to their antigenic power.

It was our normal practice to transplant test homografts of skin 3–5 days after the injection of the substances which were being examined for their antigenic power. A delay of 15 days allowed the state of immunity to become significantly weaker, and a delay of 30 days allowed it to fall to a level that was sometimes inappreciable in our test system.

The immunity produced by 'dead' preparations is therefore of much shorter tenure than that produced by 'solid' living grafts, such as skin homografts themselves[3]; we have yet to examine the duration of the immunity produced by the intraperitoneal injection of equivalent doses of dissociated living cells.

We have not yet succeeded in making active nuclear or disintegrated preparations from embryonic tissue. Yet there can be little doubt that the antigenic substances are fully developed long before birth, for the injection of so few as 5–10 m cells from 11½–12½ day mouse embryos caused the total breakdown, within six days, of test homografts of skin transplanted three days later. (The embryos were at a stage of development about equivalent to that of a 3 to 4-day chick.) It is known that, of the nine days that passed between the injection of the embryonic cells and the final scoring of the test homografts, about six days are required for the machinery of immunization to go into action and take effect; in the remaining balance of time, about three days, it is scarcely possible that the embryonic cells injected into adult mice should have advanced to the stage of development not normally achieved until birth. We may therefore take it that the transplantation antigens in mice are fully developed well before birth.

5. Action of Enzymes on Nuclei

Our study of the action of enzymes on nuclei has been complicated by their instability to mere incubation in 'physiological' media for 60 min. at 37° C. With rare and still unexplained exceptions, however, spleen nuclei, or the clean nuclear fibres prepared by the direct disintegration of spleen cells in normal citrate saline (sub-heading 2), were stable to 60-min. incubation in Ringer phosphate or Ringer bicarbonate (0·015*M* sodium bicarbonate, 0·001*M* disodium hydrogen phosphate) over the *p*H range 6·8–7·4; incubation at *p*H 5·5 (0·1*M* acetate buffer, 0·10*M* sodium chloride) or at *p*H 6·0–6·2 (phosphate buffer) was damaging but not totally destructive. On the other hand, kidney and thymus nuclei have given exasperatingly variable results, antigenic activity being sometimes lost after only 30-min. incubation. (This does not apply to kidney nuclei incubated with trypsin: see below.) The incubation of clean spleen nuclei deliberately contaminated with heavy inocula of kidney cytoplasm or red cells showed that these contaminants of kidney nuclear preparations were not to be blamed for their instability. We believed at one time that magnesium ions might be harmful—it was thought necessary, in certain experiments with desoxyribonuclease, to raise the concentration of magnesium chloride from 0·0025*M* to 0·10 or 0·0125*M*—but later experiments showed that this could not have been the whole explanation. The cause of the instability of kidney nuclei therefore remains obscure; if it is of enzymic origin, the enzymes must be presumed to be intranuclear.

For these reasons, our critical tests of the action of ribonuclease and desoxyribonuclease were made on spleen nuclei.

Ribonuclease. The incubation of spleen nuclei 100 m/ml. for 60 min. at 37° C. with ribonuclease 0·1 mgm./ml. in Ringer phosphate had no perceptible effect on their antigenic power; nor did the deliberate contamination of spleen nuclei with kidney cytoplasmic particles cause them to become vulnerable to the action of ribonuclease.

Trypsin. The gross anatomical action of trypsin on naked nuclei suspended in Ringer phosphate was spectacular: their membranes were disrupted almost instantly, and within three or four minutes at room temperature the preparation passed through a rigid fibro-gelatinous phase to form a largely clarified but only moderately viscous solution containing small quantities of miscellaneous debris in suspension.

The tryptic digestion of spleen or kidney nuclei (100 m/ml.; trypsin 0·1 mgm./ml.) in Ringer phosphate did not destroy and did not as a rule perceptibly impair their antigenic power; moreover, the serial sampling of digests in which the action of the enzyme had been stopped by adding trypsin inhibitor after 10, 20, 40 and 60 min. revealed no progressive deterioration as incubation went on. It should be observed, however, that this resistance to tryptic digestion was true *only* of nuclei prepared in the calcium-containing media which we have invariably used. Tryptic digestion of the nuclear fibres produced by the direct disintegration of spleen cells in normal citrate saline led to the formation of an almost unmanageably viscous solution with little or no antigenic power.

Desoxyribonuclease. Spleen nuclei 50 m/ml. were incubated for 60 min. at 37° C. with desoxyribonuclease 0·1 mgm./ml. in Ringer bicarbonate at a *p*H maintained between 6·8 and 7·2 throughout. After incubation, the nuclei, now translucent and grossly contracted, were spun down, resuspended in half the original volume of fluid, and injected intraperitoneally at a dosage of 1·0 ml. (100 m nuclei) per mouse. Under these conditions the antigenic power of the nuclei was completely or all but completely destroyed; control nuclei, receiving exactly parallel treatment in media lacking desoxyribonuclease, retained their antigenic power. Three points deserve special mention: (*a*) Ringer bicarbonate was used for these experiments because the weak buffer of Ringer phosphate could not prevent the *p*H of the incubation medium from falling precipitously to 6·2 or 6·4, that is, to a damaging level of acidity; (*b*) raising the concentration of magnesium chloride from 0·0025*M* to 0·0125*M* was not advantageous and might have been harmful; and (*c*) the use of lower concentrations of desoxyribonuclease or higher dosages of injected nuclei obscured the otherwise very sharp distinction between nuclei treated with desoxyribonuclease and their controls.

6. Solubility of the Antigens

It is implicit in all our methods of preparation that the antigenic substances, as they occur in their native form in nuclei or nuclear fibres, are insoluble in salt solutions of physiological strength (about 0·15*M*). The present section, an interim report on experiments still in progress, deals with their solubility in water and in strong solutions of salt.

Solubility in water. Spleen cells were prepared for aqueous dissolution as follows. The cells were expressed into, and washed in, buffered normal saline, and the spun sediment was resuspended in normal saline, to which three volumes of water were then added. The osmotically swollen cells were spun down, the pink supernatant fluid was rejected, and the somewhat gelatinous sediment was worked up in water with a coarse pipette. This aqueous suspension was exposed to ultrasound for 60 sec., whereupon most of the substance of the cells passed into a faintly opalescent and only moderately viscous solution. Matter in suspension was removed by

centrifugation at 2,000 *g*. The supernatant fluid, injected in a dosage equivalent to 250 mgm. fresh splenic tissue per mouse, was highly active. When sodium chloride was added to restore its concentration to 0·15*M*, the bulky precipitate so formed was almost completely inactive: the supernatant fluid retained nearly all the antigenic power of the original solution. When, however, calcium chloride was added to bring its final concentration to only 0·0025*M*, the precipitate contained much the greater part of the antigenic matter, though some still remained in the supernatant fluid. It is evident, therefore, that the precipitates formed by adding sodium chloride and calcium chloride to aqueous cellular solutions have different properties. The precipitate formed upon the addition of calcium chloride is insoluble in water[11]; so also are nuclei prepared, as ours have been, in calcium-containing media.

We have had less satisfactory results with aqueous extracts of the washed nuclear fraction of kidney-cell disintegrates, prepared by a variant of the methods reintroduced by Stern[12].

Aqueous solutions clarified by centrifugation at 25,000 *g* for 30 min. are antigenically inactive, although they still contain matter precipitable by the addition of sodium chloride or calcium chloride.

Solubility in strong salt solutions. The antigenic power of nuclei prepared in calcium-containing media was reduced to about 25 per cent of its original value by extraction in molar salt solution; it could be completely abolished by prolonged extraction in 2*M* sodium chloride. In this sense, which is subject to obvious reservations, the antigens may be said to be soluble in strong solutions of salt. When 2*M* salt extracts of nuclei were clarified by centrifugation at 25,000 *g* for 30 min. and then diluted with 10 vol. water, the histone nucleate fibres so formed were found to be totally inactive, whether injected intraperitoneally or intravenously, or in their original or in a finely divided state. The same was true of the much more highly fibrous preparations of histone nucleate made from nuclear fractions which had at no time been exposed to bivalent metallic ions. Histone nucleate fibres of either origin were very rapidly digested by trypsin[13] to form immensely viscous solutions containing protein cleavage products and the sodium salts of desoxyribonucleic acids. Injected in high concentrations, before or after precipitation with ethanol, these crude preparations slightly but perceptibly impaired the homograft reaction, in the sense that test homografts transplanted on the same occasion (or two days beforehand, or three to nine days later) survived slightly longer than on normal mice. In lower dosages the fresh crude digests have repeatedly but not consistently been found to exercise an immunizing action of marginal intensity. In three experiments out of four, however, nuclei first exhausted by salt extraction and then mixed with crude DNA-containing digests blocked with trypsin inhibitor produced an immunity about equal to that elicited by 25 m fresh nuclei[14].

7. Nature of the Antigenic Substances

The antigens responsible for skin transplantation immunity in homografts exchanged between *A* and *CBA* mice comprise at least 10–15 genetically distinguishable substances[1]. They are wholly confined to the nuclei of cells. In their native form they are insoluble in physiological (0·15*M*) salt solutions, but they are soluble in water, and may be precipitated therefrom by the addition of calcium chloride. The antigenic activity of nuclei can be weakened by extraction in *M* sodium chloride solution and abolished by extraction in 2*M* sodium chloride. The antigens are destroyed by digestion with desoxyribonuclease but not by digestion with ribonuclease or (under the conditions described in the text) with trypsin. They are highly unstable: in salty media, though somewhat less effectively in sucrose solutions, they are destroyed by freezing and thawing, by drying from the frozen state, and by heating to 48·5° C.

Three other properties of the antigens should be taken into consideration before passing judgment on their chemical nature: (*a*) an analysis based on the principle of immunological tolerance[4] has shown that the antigens responsible for skin transplantation immunity, so far from being peculiar to skin, are uniformly represented in all the living tissues of a single individual; (*b*) although living whole blood can elicit skin transplantation immunity (or tolerance[4]), its power to do so resides in the leucocyte fraction and is lacking from red cells, plasma and platelets[15]. (Platelets are of cytoplasmic origin and contain no desoxyribonucleic acid[16].) Finally, (*c*), the antigens are developed well before birth.

So far as we are aware, only one hypothesis can accommodate these findings: that the antigenic substances responsible for skin transplantation immunity are desoxyribonucleoproteins endowed with antigenic and therefore with genetic specificity. This hypothesis is made *likely* by our evidence, but the evidence falls short of proof. In particular, the properties of aqueous solutions of the antigenic substances, and of the precipitates formed from them by adding sodium chloride and calcium chloride, require further investigation. (It should not be forgotten that these aqueous solutions, particularly those prepared by ultrasonic methods, may be quite extensively degraded[17].)

If the antigens are indeed desoxyribonucleoproteins, they are almost certainly not, or not merely, nucleohistones. It is not, of course, to be expected that the genetically specific structure of a nucleoprotein should be even approximately reproduced when histone chloride and the sodium salts of desoxyribonucleic acids reassociate to form histone nucleate after diluting their solutions in strong salt[12,13,15]. On the other hand, we were surprised to find that the nucleohistone formed by adding sodium chloride in 0·15*M* concentration to an antigenically active aqueous solution was itself inactive. The precipitate formed by adding calcium chloride in a concentration of 0·0025*M* was strongly active, and the importance of the calcium ion made itself apparent in other ways; for example, in making the native nuclear antigens (*a*) stable to tryptic digestion, and (*b*) partly though not completely resistant to extraction by molar salt solution. The physical instability of the antigens suggests lipoprotein attachments, and it may well be, as several authors have suggested, that the nucleic acid is attached not merely to histone but also to the residual lipoid-containing 'acid' proteins of the nucleus as well[18]—an attachment in which bivalent metallic ions could well play an important part, for calcium[18] and magnesium[11] are both present.

There are well-recognized theoretical reasons for believing that the genetic and antigenic specificity of a nucleoprotein should reside in the desoxyribonucleic acid moiety, and in due course we hope to find out for certain whether or not this is really so. For this purpose it will clearly be necessary to devise

some method for recombining desoxyribonucleic acid with nuclear protein which is much more efficient and reproducible than that described under sub-heading 6. It would be idle to discuss the origin of the weak antigenic action sometimes exercised by crude digests of histone nucleate fibres containing desoxyribonucleic acid until the possibility that it is due to desoxyribonucleic acid itself can be confirmed. At present it seems more likely to be due to the presence of small quantities of undissociated nucleoprotein—if indeed it is not due to substances of some altogether different kind.

Naked nuclei are only one-fifth to one-tenth as active as living cells in provoking transplantation immunity, and aqueous solutions of the antigens are weaker still. Part of the difference, perhaps a large part, can be attributed to the fact that they must be specially vulnerable to the endogenous desoxyribonucleases of the mice into which they are injected.

In view of the possibility that the antigenic substances are desoxyribonucleoproteins, we attach special importance to the experiments which have led us to believe that they are continually emitted by normal living cells[4].

8. Bearing on the Immunology of Transplantation

One important, if perhaps merely apparent, inconsistency between our work and that of others must now be resolved. It is known from a great wealth of genetical evidence that the transplantation antigens of an individual mouse comprise a multiplicity of substances which are intimately gene-controlled[20]. Some of the more important 'histocompatibility genes' which have been identified by genetical methods are already known from the pioneer work of Gorer[21] to determine a special system of iso-antigenic substances, hereafter '*H*-antigens' for short. *H*-antigens do not elicit skin transplantation immunity, and they are physically quite distinct from the nuclear antigens which we have just described. They are present in red cells and in cytoplasm; in mice, they are not formed until after birth[22]; and they are comparatively stable: lyophilized tissues, for example, are rich in *H*-antigens, though they do not elicit skin transplantation immunity. *H*-antigens are recognized and serologically labelled by their power to elicit the formation of orthodox serum iso-antibodies which can agglutinate homologous red cells, but there is clear evidence that these antibodies are not the effectors of transplantation immunity, at least of 'solid' grafts[23]. (It should be added that Bollag[24] has identified a system of iso-antigens in rabbits by purely serological methods. They cannot yet be classified with certainty; but their distribution, stability and solubility make it quite possible that they are nucleoproteins.)

The apparent paradox, then, is this: that the same histocompatibility alleles determine the formation *both* of the cytoplasmic *H*-antigens *and* of the nuclear antigens that are the prime movers in transplantation immunity. We can therefore only suppose that the two classes of antigens stand to each other in the relationship of secondary and immediate gene products; indeed, if our interpretation of their chemical nature is correct, the nuclear antigens consist of chromosomal matter. Certain questions suggest themselves immediately. Can the nuclear antigens elicit the formation of serum antibodies of the same kind as those produced by *H*-antigens, or, if not, can they specifically absorb the serum antibodies which *H*-antigens have formed? Again, how does it come about that homografts killed by, for example, lyophilization, and incapable of causing transplantation immunity, may yet elicit a brisk inflammatory response when implanted into animals which have been immunized beforehand by living homologous cells?[25] These and other questions[26] belong to the agenda of future research.

Summary

Cells totally disintegrated by ultrasonic irradiation retain their power to elicit skin transplantation immunity in mice. The substances responsible for this property reside wholly in the nuclei of cells, and are developed well before birth. They are inactivated by lyophilization, by freezing and thawing, and by heating to 48·5° C. They are inactivated by digestion with desoxyribonuclease but not with ribonuclease or, in certain stated circumstances, with trypsin. They are insoluble in salt solutions of physiological strength, but are soluble in water, from which they may be precipitated in an active form by the addition of calcium chloride; and they are extracted from nuclei by treatment with 2*M* sodium chloride solution. These facts, together with cognate evidence from other sources, make it probable that the antigens responsible for skin transplantation immunity are desoxyribonucleoproteins.

[1] Barnes, A. D., and Krohn, P. L. (personal communication).
[2] Billingham, R. E., Brent, L., Medawar, P. B., and Sparrow, E. M., *Proc. Roy. Soc.*, B, **143**, 43 (1954).
[3] Billingham, R. E., Brent, L., and Medawar, P. B., *Proc. Roy. Soc.*, B., **143**, 58 (1954).
[4] Billingham, R. E., Brent, L., and Medawar, P. B., *Phil. Trans. Roy. Soc.*, B, **239**, 357 (1956).
[5] The important discovery that grafts from male mice are eventually rejected by females of the same strain—see Eichwald, E. J., and Silmser, C. R., *Transplantation Bull.*, **2**, 148 (1955)—does not affect the design of the present experiments.
[6] Compare Mizen, N. A., and Petermann, M. L., *Cancer Res.*, **12**, 727 (1952).
[7] We are obliged to Dr. A. S. McFarlane for allowing us to use the instrument in the Biophysics Department of the National Institute for Medical Research.
[8] See, for example, Gale, E. F., and Folkes, J. P., *Nature*, **173**, 1223 (1954).
[9] Schneider, W. C., and Petermann, M. L., *Cancer Res.*, **10**, 751 (1950).
[10] Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.*, **172**, 619 (1948).
[11] Gilbert, L. M., Overend, W. G., and Webb, M., *Exp. Cell Res.*, **2**, 349 (1951).
[12] Stern, K. G., and Davis, S., *Fed. Proc.*, **5**, 156 (1946). Stern, K. G., Goldstein, G., Wagman, J., and Schryver, J., *Fed. Proc.*, **6**, 296 (1947). See also Carter, R. O., and Hall, J. L., *J. Amer. Chem. Soc.*, **62**, 1194 (1940).
[13] Cohen, S. S., *J. Biol Chem.*, **158**, 255 (1945).
[14] Compare the experiments of Gale and Folkes (ref. 8) and of Allfrey, V. G., and Mirsky, A. E., *Nature*, **176**, 1042 (1955).
[15] Unpublished observations.
[16] Greene, R. W., *Proc. Soc. Exp. Biol. Méd. N.Y.*, **87**, 412 (1954). Humphrey, J. H., *Nature*, **176**, 38 (1955).
[17] Shooter, K. V., Davison, P. F., and Butler, J. A. V., *Biochim. Biophys. Acta*, **13**, 192 (1954).
[18] Bernstein, M. H., and Mazia, D., *Biochim. Biophys. Acta*, **11**, 59 (1953).
[19] Wang, T. Y., Mayer, D. T., and Thomas, L. E., *Exp. Cell. Res.*, **4**, 102 (1953). Engbring, V. E., and Laskowski, M., *Biochim. Biophys. Acta*, **11**, 244 (1953).
[20] Review by Snell, G. D., *J. Nat. Cancer Inst.*, **14**, 691 (1953). Amos, B., Gorer, P. A., and Mikulska, Z. B., *Proc. Roy. Soc.*, B, **144**, 369 (1955).
[21] Gorer, P. A., *J. Path. Bact.*, **44**, 691 (1937); **54**. 51 (1942); *Brit. J. Cancer*, **2**, 103 (1948); see also ref. 20.
[22] Mitchison, N. A., *J. Genet.*, **51**, 406 (1953).
[23] Mitchison, N. A., and Dube, O. L., *J. Exp. Med.*, **102**, 179 (1955). Algire, G. H., Weaver, J. M., and Prehn, R. T., *J. Nat. Cancer Inst.*, **15**, 493 (1954). Weaver, J. M., Algire, G. H., and Prehn, R. T., *J. Nat. Cancer Inst.*, **15**, 1737 (1955).
[24] Bollag, W., *Experientia*, **12**, 57 (1956).
[25] Darcy, D. A., *J. Path. Bact.*, **70**, 143 (1955).
[26] We discuss the bearing of this work on the phenomenon of 'enhancement' in *Transplantation Bull.*, **3**, 84 (1956).

OBITUARIES

Sir Harold Scott, K.C.M.G.

Sir Harold Scott, who died at Braintree on August 6, shortly after his eighty-second birthday, was widely known as a pathologist, a historian of tropical medicine, and an editor. After taking medical qualifications in 1897, he served in the South African War, returned to England and entered general practice and then went to Jamaica as Government pathologist. The First World War brought him once more into the R.A.M.C., and after its conclusion he became Milner Research Fellow at the London School of Hygiene and Tropical Medicine, where he elucidated the life-history of *Hymenolepis*. He was then appointed Government pathologist at Hong Kong, retaining this post for several years until compelled to retire through ill-health. Later he became pathologist to the Zoological Society of London, medical secretary of the Colonial Medical Research Committee, and in turn assistant director (1930) and director (1935) of the Bureau of Hygiene and Tropical Diseases, until his retirement in 1942.

As a pathologist he published a large number of papers and monographs on tropical subjects—particularly on the vomiting sickness of Jamaica, the central neuritis of Jamaica, and on tuberculosis in man and lower animals (based on his experience in Hong Kong and at the Zoo). He wrote several books, including "Some Notable Epidemics" (1934), but his most important literary work was his great "History of Tropical Medicine" (1939), which has been recognized throughout the world as a masterpiece. At the Bureau of Hygiene and Tropical Diseases he edited the *Tropical Diseases Bulletin* and the *Bulletin of Hygiene*, and it was under his direction that the *Bulletin of War Medicine* was created. He was appointed C.M.G. in 1935 and K.C.M.G. in 1941.

Scott was for many years associated with the Royal Society of Tropical Medicine and Hygiene, of which, to his great pleasure, he was elected president in 1943.

One of his most outstanding characteristics was a capacity for hard, sustained and conscientious work. He was a man of high intelligence and quick decision, and he worked rapidly, leaving himself ample time for the voracious reading which was his chief relaxation. He was a great buyer of books on all kinds of subjects, and accumulated a large and varied library, particularly rich in historical works. He loved his canaries and budgerigar, and the animals at the Zoo, and this no doubt stimulated his interest in the immense subject of the animal reservoirs of human disease, so important in tropical medicine.

Scott was a delightful companion, full of humour, which on occasion could be pointed, but which was modified by a rather old-world courtesy. Physically he was slight, good-looking, and always immaculately dressed. He moved with ease and assurance among the leaders of his profession and made many friends. His first wife died in 1933; his second wife, Eileen Anne, daughter of the Rev. R. P. Prichard, survives him.

Charles Wilcocks

Dr. E. Leonard Gill

E. Leonard Gill died in Cape Town on July 5. He was born in Reigate and after attending a Friends school, entered Owens College, Manchester, graduating in 1899. He then became a member of the museum staff at Manchester and so gained experience in museum arts and management. Later, he became curator of the Hancock Museum, Newcastle upon Tyne. Here he was responsible for large, important collections of fossils, birds and marine invertebrates, as well as the general contents of an old-established natural history museum. The maintenance of this large building, very old-fashioned in its design and furnishing, also fell to him, and the funds of the Society which owned it were, even then, inadequate.

Gill served in the Friends Ambulance throughout the First World War, returning to the Museum, when he began to modernize some exhibits. In 1922 he accepted a post in the Royal Scottish Museum; but in 1924 he became director of the South African Museum, Cape Town.

In the seventeen years he spent in this post, Gill revolutionized the Museum; its size was nearly doubled, the exhibition galleries were modernized and enlarged, and the scope of the Museum was extended to cover not only important South African antiquities, but also some art collections. Gill wrote descriptive labels, in Africaans as well as English, which brought the Museum into much closer touch with the general public, and increased this contact by circulating specially made museum cases to schools.

Gill published a few useful papers on fossil fish; but his chief interest was in birds. On his first visit to the Museum in Cape Town, he found on his table a dead bird just picked up and brought in. On its leg was a ring which Gill himself had placed there at a nest in the Farne Islands. Gill found that there was no small cheap book about South African birds, so that it was impossible for any ordinary man to know them, and thus to observe them intelligently. Hence he wrote an inexpensive book with many small but admirable figures drawn by Gill himself and his artist sister. This book was most useful, not only in South Africa but even farther north, and it has gone through several editions.

Gill added to the general interest in natural history, by newspaper articles in Africaans as well as English, and by lecture talks. Also he founded the South African Ornithological Society, which recently celebrated its silver jubilee.

Gill was a man of unusual character, willing to take great trouble to help anybody, friendly and helpful to everyone.

D. M. S. Watson

Mr. John A. Marsh

Through the death of John Anderson Marsh, which occurred on July 1, the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) has lost a greatly valued chief technician who had given it his loyal service for more than forty-six years. Appointed to the Institute on its foundation in 1909, he then worked for its first director, Alexander Paine, and for Casimir Funk and Jack (later Sir Jack) Drummond, at that time engaged in investigations of the 'vitamines' and of the amino-acid composition of tumours, respectively. Under his later directors, Archibald Leitch and Sir Ernest Kennaway, he played an essential part in assisting their contributions in the field of chemical carcinogenesis, first in studies with coal-tar, and later in the great development of the cancer-producing hydrocarbons.

A man of wide interests and much kindliness, Marsh inspired friendship in all his colleagues, not least in the numerous workers from abroad whom he had helped over the years, and who mourn him equally with ourselves. He had seen great changes in research, and throughout his long service at all times embodied the true worth of the chief technician. Both for his unique contribution and for his personal qualities, he will be ever gratefully remembered by all who knew him. ALEX. HADDOW

NEWS and VIEWS

New President of the British Association: Prof. P. M. S. Blackett, F.R.S.

AT the meeting of the General Committee of the British Association for the Advancement of Science held in Sheffield on August 29, Prof. P. M. S. Blackett was elected to succeed Sir Raymond Priestley as president. Prof. Blackett was born in 1897 and educated for the Navy at Osborne and Dartmouth. After war service during 1914–18 he studied at Cambridge, where he took Part I of the Mathematical Tripos and Part II of the Natural Sciences Tripos in physics. He then worked under Rutherford in the Cavendish Laboratory until 1933. From 1933 onwards he has been successively professor of physics at Birkbeck College, London, University of Manchester and the Imperial College of Science and Technology, London. He received a Royal Medal from the Royal Society in 1940 and was awarded a Nobel Prize in 1948.

Prof. Blackett and those working with him have made significant contributions to three main fields of physical discovery: the interaction with matter of fast particles from radioactive sources, the nature of the particles in the cosmic rays and the magnetism of the Earth. During 1921–31 he was chiefly occupied with the development and operation of automatic Wilson cloud chambers and their application to the precise measurement of the parameters involved in collisions between alpha-particles and atomic nuclei. By painstaking work he showed that very infrequent inelastic collisions with nitrogen produced the then unknown isotope oxygen-17 by the capture of the alpha-particle and the ejection of a proton. In this period also he verified, with Champion, the Mott theory of the wave-mechanical diffraction scattering of slow alpha-particles. From 1931 onwards he applied and extended the cloud chamber technique to the study of the collisions involved in the cosmic rays, establishing (with Occhialini) the existence of nearly equal numbers of positive and negative electrons. This confirmed Anderson's discovery of the positive electron a few months before and extended it by giving strong evidence for actual pair production. In addition, the 0·5-MeV. scattered gamma-rays were identified as arising from the annihilation of positive and negative electron pairs. With a large magnet and a new counter-controlled cloud chamber, Blackett and his colleagues proceeded to study in more detail the nature and the energy spectrum of cosmic ray particles. Much valuable work on cosmic ray showers was carried out under his direction, and he himself explained the negative temperature coefficient of the cosmic rays in terms of the decay of the μ-meson.

These detailed studies led to a discovery which opened up the entirely new field of 'strange' particles when Rochester and Butler in Blackett's laboratory in 1946 found the first neutral V-particles and in 1947 the first charged V-particles. The identification by the Manchester team of V^0 decay into a proton and a pion and of the probable division of a V^1 particle into two pions, together with the best determination to date of the life-times of V^0 and V^1 neutral particles, are outstanding further discoveries. These were made possible by Blackett's energetic action in moving the equipment to the observatory of the Pic du Midi. From 1947 onwards he interested himself in the possibility, originally discussed by Schuster, of a 'fundamental' connexion between the angular momentum of a large rotating body, such as the Earth, and its magnetic field. Although experiments conducted and suggested by him gave no support to this idea, the sensitive magnetometer designed for this work has been used by him and by Runcorn at Cambridge in remarkable studies of palæomagnetism which give promise of throwing new light on polar wandering and continental drift.

Chemistry in Swansea: Prof. C. H. Hassall

PROF. C. H. HASSALL, professor of chemistry in the University College of the West Indies, has been appointed to the chair of chemistry in University College, Swansea (University of Wales), in succession to Prof. C. W. Shoppee, who has accepted the chair of organic chemistry in the University of Sydney, New South Wales, Australia. Prof. Hassall graduated in the University of New Zealand in 1941 and after war service went with a senior studentship of the 1851 Exhibition to Cambridge, and carried out research in organic chemistry with Sir Alexander Todd. In 1948 he accepted the chair of chemistry in the young University College of the West Indies, where he has been responsible for building up a new department, involving the design of laboratories, recruitment of staff and the development of teaching and research. During this period he has also managed to visit Harvard, where he worked in Prof. R. B. Woodward's laboratory for a time, and a number of university departments and industrial research laboratories in England working on organic natural products. These activities explain in part the close links which Prof. Hassall has formed between his department and industry in the West Indies and with several pharmaceutical firms in Europe. He has served as the United Kingdom representative on the Caribbean Research Council and is a member of the advisory committee of the Institute of Social and Economic Research, Jamaica. His research work has been mainly in the field of the structural chemistry of organic compounds of biological activity such as steroids, polypeptides and antibiotics.

Weather Forecast Service in Britain by Telephone

THE automatic weather forecast service by telephone which came into operation in Britain at midnight on March 4 has been a great success. In the first month 561,377 calls were received from the public, a number far exceeding the number made to the London Forecast Office in any month; the number of calls to the Office during February was 17,128. This experience is similar to that in other countries. The number in the first month was

probably boosted by mere curiosity, for the number of calls in July was 339,384. Clearly the automatic system provides an 'on demand' weather forecast service quite beyond the capacity of a forecast office answering direct inquiries. The mechanical arrangements of the forecasting service are described by W. R. Hanson in the July number of the *Meteorological Magazine*. The forecasts are prepared in the Meteorological Office and recorded on tape at telephone exchanges of the General Post Office. They do not, as has been the arrangement elsewhere, consist of the nearest applicable stock phrases, but are compiled to express the forecaster's opinion as nearly as possible within the limits of the time allowed—which must be at most 30 sec. The forecast can be revised at any time, and there is provision for automatic switching in of a second tape if the first breaks down and for keeping the service going during recording of a new forecast.

Nuclear Research Reactor at the Massachusetts Institute of Technology

The National Science Foundation, Washington, D.C., has made a grant of 500,000 dollars to the Massachusetts Institute of Technology towards the construction of a nuclear research reactor, plans for which have been under way since 1952. The remaining cost, which will be at least 2,000,000 dollars, will be borne out of funds raised by Massachusetts Institute of Technology alumni and by a grant from the Rockefeller Foundation. The reactor will use enriched uranium-235 as fuel, and will be cooled and moderated by heavy water. The main core will be surrounded by a graphite reflector and housed in an air-tight building with a dome-shaped roof, the site for which is 136 Albany Street, Cambridge, Mass. Construction is already under way and is expected to be completed in the autumn of next year. The design of the reactor has been chosen to give the maximum degree of safety together with facilities for a large number of experiments in nuclear physics, which will be the principal function of the reactor. However, it will also be made available for research in solid-state physics, food technology, genetics, chemical and nuclear engineering, and medicine. The whole project is under the general direction of Dr. Manson Benedict, professor of nuclear engineering in the Department of Chemical Engineering. Dr. T. J. Thompson, formerly chairman of the Design and Construction Committee of the Omega West Reactor of the Los Alamos Scientific Laboratory, has designed the reactor and will supervise its construction.

Gwerin : a New Journal of Folk-lore

A new journal of folk-lore, *Gwerin* (Welsh for 'folk'), has been inaugurated, and the first number appeared in June. The forty-eight pages of the number contain three main articles, the first on how certain straw-rope granaries near Cork are made, a second on some Hebridean traditions, and a third on the manufacture in Suffolk of wooden rakes and scythe sticks. It is not easy to make descriptions of how things are constructed readily intelligible, and it is virtually impossible to make them exciting. Therefore, if the new journal is to survive, one might suggest that plenty of room should be set aside for the legend and fairy-tale side of the subject, which always has a great appeal. But constructional descriptions should be recorded somewhere even if only the accounts are deposited in some recognized museum. The straw-rope granaries make an interesting article, and it must be news to many that rakes and scythe sticks were a special feature of rural industries in Cambridgeshire and the neighbouring counties of Suffolk and Bedfordshire. It is good to know that story-telling and traditional music are still popular in the Hebrides and that they have not been ousted by the modern, 'popular' entertainment that pours forth over the radio and television and from so many printing-presses and film studios. There is, indeed, room for a journal devoted to folk-lore, and all will wish the new venture every success.

Makers of Modern Science

The series of ten articles, each devoted to a brief description of one of the great prime movers in the development of science, which appeared originally in *The Times Educational Supplement*, has now been reprinted and issued as an attractive booklet, entitled "Makers of Modern Science" (pp. 44+10 plates. London: The Times Publishing Co., Ltd., 1956; 2*s*. 6*d*.). Each article is accompanied by a full-page photograph of its subject. The aim of the contributors, present-day eminent men of science, is to write about their subjects in a way which would not appear absurdly elementary to specialists in science and yet which would not be too repellently difficult for specialists in humanities. It is hoped that the articles will act as a bridge of general conversation between arts men and scientists. Except for Louis Pasteur and Charles Darwin, the prime movers are physicists or chemists who laid the foundations for our present-day knowledge of the structure of matter and of the atom in particular—Galileo, Newton, Dalton, Faraday, Mendeleev, J. J. Thomson and Rutherford. The late Dr. Sherwood Taylor, who took a large part in the preparation of the series, contributes the article on Galileo, and this represents one of the last writings of this distinguished historian of science. Sir Lawrence Bragg is the author of the article on Faraday; Dr. Charles Singer on Pasteur; Prof. J. R. Partington on Mendeleev; Sir George Thomson on his father; and Prof. P. M. S. Blackett on Rutherford.

Basic Concepts and Methods of Psycho-analysis

The centenary of Freud is commemorated in a recent number of the *British Journal for the Philosophy of Science*, which contains seven articles that attempt, with considerable though varying success, to clarify some of the basic concepts and methods of psycho-analysis. Two contributions state in very clear terms the theoretical and practical difficulties that arise from the over-determination of mental phenomena—that is, the fact that many of these data are explicable in terms of different, and incompatible, laws and hypotheses. Similar problems are, of course, encountered in other fields of science, so that this number of the *Journal* is of quite general interest.

Commonwealth Fund Fellowships for Advanced Study and Travel in the United States

A number of Commonwealth Fund fellowships are again being offered by the Commonwealth Fund, New York, to British subjects for study and travel in the United States. All expenses of travel, study, and living will be met, with some adjustment of stipends for married men. The fellowships which are open to British men or women who have not previously worked or studied for more than a few

months in the United States are in six categories as follows. *General:* twenty fellowships to graduates of universities in Great Britain or Northern Ireland; candidates must be available in London for interview in March 1957; age, 23–32; tenure, 12–21 months; applications by December 15. *Home Civil Service:* five fellowships to permanent members in the higher ranks of the Civil Service in Great Britain, three for the administrative grades and two for the scientific and professional grades; age, preferably less than 40; tenure, 9–15 months; applications by December 10. *Australian and New Zealand Civil Services:* five fellowships to Civil servants in the Governments of Australia and New Zealand; age, less than 40; tenure, 9–15 months; applications (to Dr. H. Carlyle Forster, Box 4079, Melbourne, and the Public Service Commission, Wellington, N.Z., respectively) by November 1. *Oversea Civil Service:* two fellowships to Civil servants in the Governments of British Colonies, Protectorates, and Trust Territories; age, less than 40; tenure, 9–15 months; applications by November 17. *Journalism:* three fellowships to journalists practising in Britain and engaged on the opinion-making or broadly editorial side of their profession; age, 23–35; tenure, 9–15 months; applications by December 31. *American Studies:* four fellowships to faculty members appointed to or holding posts in American studies in universities in Britain; candidates to be nominated by their universities; tenure, 4–15 months (these fellowships are not limited by previous study in the United States). Application forms and further information can be obtained through British universities or through Government departments, or from the Warden, Harkness House, 35 Portman Square, London, W.1.

Seventh International Astronautical Congress

THE Seventh International Astronautical Congress, sponsored this year by the Associazione Italiana Razzi (Piazza S. Bernardo n. 101, Rome, Italy), will be held in the Congress Palace, Rome, during September 17–22. A total of forty-two papers will be presented at the Congress, as follows: United States, 18, including six on the Vanguard satellite and related problems; Italy, 8; Germany and Austria, 4 each; Holland, 2; and Great Britain, Holland, Egypt, Poland, Sweden and Argentina, 1 each. Arrangements have been made for simultaneous translation service in Italian, French, English and German. Further information can be obtained from the British Interplanetary Society, 12 Bessborough Gardens, London, S.W.1.

Announcements

THE thirty-second meeting of the Australian and New Zealand Association for the Advancement of Science will be held in Dunedin, New Zealand, during January 16–23, 1957, under the presidency of Sir Macfarlane Burnet. Enrolment forms, to be completed by December 12, and further information can be obtained from the honorary local secretary, Prof. J. R. A. McMillan, Science House, 157 Gloucester Street, Sydney, N.S.W. Those seeking accommodation should complete the necessary form and return it by October 31 to Dr. R. E. Corbett, Department of Chemistry, University of Otago, Dunedin, N.I., N.Z.

THE Scottish Group of the Nutrition Society will hold a symposium in Dundee on September 29 on "Nutrition in Pregnancy". The papers to be read will deal with both human and animal nutrition. Those interested should write for further particulars to Dr. J. Davidson at the Rowett Research Institute, Bucksburn, Aberdeenshire.

THE fourth in the series of international conferences on Alpine meteorology will be held in Chamonix during September 17–19. The topics to be considered will be: precipitation in mountain areas; the types of weather in the Alpine region and their classification; radiation in mountain areas; and the bioclimatology of the Alps. Further information can be obtained from the Secretariat of the Congress at 72 rue Pasteur, Lyons, Rhône.

THE Textile Institute will hold a conference in Zurich during September 13–17 at which the subject of "Static Electricity in Textiles" will be discussed. This will be the second in the Institute's series of overseas conferences, and more than a hundred textile experts and scientists from Britain will join about the same number from other parts of Europe and from North America. Further information can be obtained from the Development Officer of the Textile Institute, 10 Blackfriars Street, Manchester 3.

A SYMPOSIUM on "Recent Studies in Yeast and their Significance in Industry", jointly arranged by the Dublin and District Section, the Microbiology Group and the Food Group of the Society of Chemical Industry, will be held in the Rupert Guinness Hall, St. James's Gate, Dublin, during September 17–19. The symposium will be open to non-members of the Society on payment of a registration fee of 25*s*. Further information can be obtained from Dr. A. K. Mills, Chemist's Laboratory, Arthur Guinness Son and Co. (Dublin), Ltd., St. James's Gate, Dublin.

THE Ford Foundation has given a grant of 400,000 dollars to the European Organization for Nuclear Research to help in strengthening co-operation in nuclear physics research, primarily with the United States and with other countries not members of the Organization. The grant is to be spent over five years and will be used mainly for enabling guest professors and young scientists to work in the Organization.

THE University of Padua is offering a Gabriella Zuccari scholarship of 300,000–600,000 lire for research of at least six months duration at the Zoological Station, Naples, during 1957. The scholarship is open to any person for research in zoology, comparative anatomy, physiology or embryology. Application forms, to be returned by October 31, can be obtained from the Administrative Director of the University of Padua.

THE report for 1955 of the Chemical Research Laboratory, Teddington (pp. 88+1 plate. London: H.M.S.O., 1956; 4*s*. net), contains an interesting survey of work carried out there. Some of the activities are linked with that of the United Kingdom Atomic Energy Authority and include the extraction of uranium and other metals, the separation of rare earths, and related problems. Other work is on the preparation of semi-conductors in a very pure state, paper chromatography, and the bacterial breakdown of aromatic substances available as waste-products.

ERRATUM. In the article entitled "The Neutrino" in *Nature* of September 1, p. 446, equation 7 should read:

$$\sigma = \left(\frac{G^2}{2\pi}\right)\left(\frac{\hbar}{mc}\right)^2\left(\frac{p}{mc}\right)^2\frac{1}{(v/c)}$$

APPLIED RESEARCH AND DEVELOPMENT WITHIN THE INDUSTRIAL GROUP OF THE U.K. ATOMIC ENERGY AUTHORITY

By L. ROTHERHAM* and Dr. A. B. McINTOSH†
Industrial Group, U.K. Atomic Energy Authority

IN the United Kingdom Atomic Energy Authority, the Industrial Group is responsible for the design, construction and operation of plants for the production of fissile material and for the prototype power stations such as that at Calder Hall or Dounreay. It deals with sanctioned commitments dispersed at sites throughout the whole country, and has three main branches with clearly defined responsibilities, each under its own director. The design and construction of reactors and process plants are the responsibility of the Engineering Branch, and their operation and level of production are the business of the Operations Branch.

The Research and Development Branch deals with development work affecting the Industrial Group, and its laboratories are suitably related to geographically separated works sites. It is responsible for the removal of difficulties preventing full production in any part of a production plant, the development of works processes, the provision of information for the design of new plants and prototype reactors, and the longer-term studies arising from production and design. Close collaboration is maintained with the Research Establishment at Harwell, which undertakes part of the work.

The Branch has its administrative centre at the Industrial Group headquarters at Risley, where the headquarters of the Engineering and Operations Branches are also located. Continuous consultation is thus assured between the main centres of administration, finance, engineering and operations.

There are five Laboratories and, except for some specialized fields in which Harwell is responsible, these provide the development effort for all sanctioned construction projects. The Capenhurst Laboratories deal mainly with problems of an engineering nature, such as fluid flow and heat transfer. At Windscale, the Laboratories are largely devoted to radioactive chemistry, metallurgy and physics, arising from reactor problems at Windscale, Calder and Chapel Cross, and to the chemical problems of plutonium separation. Culcheth is responsible for the selection and development of reactor materials and for research work on the extraction of these if they are not commercially available. This is extended on a narrowed front at the Springfields Laboratory, which is responsible for the development of fuels and fuel elements. The new Laboratories at Dounreay are concerned with experimental reactor technology.

Planning and Progress of Applied Research

In the development of nuclear power there is necessarily a closer contact between scientists and engineers than in many well-established activities, because each new venture represents a major advance in technology. Applied research must be regarded as an essential complement to engineering and should be organized as such.

Both Engineering and Operations (or Works) Branches require the support of timely, authoritative and responsible scientific opinion to form the basis of major engineering and operational decisions. Decisions should not have to be made from the information nearest in character arising from a distantly related research problem. Investigations must aim at providing the precise information on the day it is required. It must be research applied to industrial needs and not pure research. Only applied research can be designed to influence commitments already made. The emphasis must always be on obtaining necessary techniques or data by a particular date. All work must therefore be planned and progressed in the same way as the Engineering Construction or Production Programmes to which it is linked.

The system of organization which has been developed begins with a study of work requirement aimed at providing the scientific personnel with a profitable programme of research and development. Such a programme, however, is no more than a declaration of intention. It must be integrated with a system of executive management which, in providing the machine to discharge the programme, preserves scientific integrity, and yet allows firm direction and control.

Many different and competing schemes may be judged as suitable for feasibility study, development or design, although restrictions may eventually be imposed due to limitations in available engineering effort. A widely dispersed research and development effort stretched over too many projects is less effective than a more concentrated attack on a narrow front. At the earliest possible stage responsible assessment must decide the most profitable direction for the application of the available applied research and development effort. The constant need for selecting only projects from which the greatest return will be obtained leads to the dual needs of strategic and tactical planning and to the development of two types of organization, functional and executive, each directly responsible to the director. The resolution of the separate points of view leads to the formulation of research policy which must conform to the overall policy of the Board of Management. The initiation, control and termination of all work necessary in the execution of this policy is effected by the wise use of delegated financial authority. The Director of Research is chairman of the Research Board formed for this purpose. The Secretary is the senior scientist in charge of central administration, and the members from the Branch are the heads of all the experimental establishments and the chief scientists responsible for functional activities. Members from outside the Branch are the Directors of Administration, Engineering, Operations, Stores and Accounts and Technical Policy or sufficiently senior deputies.

* Director of Research and Development, U.K.A.E.A. (Industrial Group).
† Head of Culcheth Laboratories, U.K.A.E.A. (Industrial Group).

A body constituted in this way brings together a group of the most senior staff appropriate to consider the action arising from all matters affecting general and technical policy, organization and finance.

The procedure adopted is for a short paper to be prepared by one of the Research Board and circulated to other members prior to debate. On the Organization and Finance side, typical subjects under constant review are methods of reporting of work, training schemes, staff structures complements and accounting procedures. On the technical side the attitude to specific research projects is clarified by debate following presentation of papers from both functional and executive points of view.

Firm direction of experimental work to a policy is assured if control is exercised on capital and operational expenditures, staff recruitment and the requisitioning, initiation, progress and termination of work.

Annual Budgets

Each year the heads of experimental establishments submit to the Research Board estimates of their capital and operational expenses for the next financial year. A total is agreed for each and the collective budget is presented by the Director to the Board of Management for approval. Ultimate acceptance and voting of funds does not constitute authority to spend.

Each proposed item of expenditure beyond a small sum must be separately justified during the financial year. The Director of Research and Development has a delegated financial authority from the Board of Management which he ordinarily exercises through the Research Board. A lower authority is further delegated to each establishment head. Beyond the delegated authority, submission must be made to the Board of Management; but no proposal will go forward without prior discussion at and support of the Research Board.

A statement of running costs under separate cost headings is available for consideration at each meeting of the Research Board. These are issued by the Accounts Branch and show the actual budgeted expenditure for the period together with the recorded and budgeted cumulative total fraction of the year's expenditure. These statements are among the most important documents for laboratory management.

Requisition, Initiation and Review of Work

A sanction procedure has been carefully worked out and coupled with a formal system of work requisition and initiation. Where a large allocation of effort is involved which is equivalent to major operational expense, a formal justification must be made as a development proposal which will briefly detail the problems, methods of approach proposed, staff utilization and associated capital expenditure according to a timed programme of the work.

Each month the Research Board has before it a statement giving all jobs due for review or completion in that month. This includes a comparison of the estimated and the recorded labour expenditure and cost. Departures from the initial timed plan in the expenditure of labour or finance or in technical progress must be accounted for to the Board's satisfaction.

A progress statement on each job in the Branch Programme is made each two months, and opportunity is afforded each member of the Research Board to raise any question regarding the work. The collected progress statements, which give for each job the object of the work, the priority, estimated effort, starting and finishing dates, review dates and brief up-to-date progress statement are forwarded for the approval of the Board of Management. This document is unique in its value to chief engineers, works general managers, and all workers in the field.

Staff

The staff position in each laboratory is considered each month and curves show how recruitment in the Branch compares with that forecast. Recruitment campaigns or embargos are approved as a result. Other aspects discussed include recruitment difficulties in particular grades or non-uniformity in duties of grades from laboratory to laboratory.

Because there is a shortage of scientists, it has been an item of policy to take positive steps to ensure the most efficient use of every member by selecting the most rewarding items of research on which to utilize available effort. Such programming of work cannot do more than indicate directions for effort. It is for executive management to form a tactical plan to ensure that each member is suitably and wholly employed.

Survey of Competitive Demands

In many fields of modern engineering and works practice, as with atomic energy, highly specialized sciences either do or should form the basis of the activity.

A design project which may begin as an idea in the Research Branch, although initiated as a result of experience or inspiration in any of the branches, becomes a sanctioned commitment only after successful study of feasibility. Each commitment sanctioned requires planning to ensure that all facets are covered so that the target date is met. Formal or informal discussion of basic technical points on which key decisions are necessary usually leads to demand for scientific investigation. Such demands compete with one another, and it is a specialized study to assess the comparative weight which should be allowed for each.

All engineering and production programmes must be interpreted so that effort can be deployed in phase. Work performed out of phase denies effort to essential and relevant studies of the moment. This timing is vital in the later allocation of laboratory priorities.

The research programme can be designed to influence the trend of development. Responsible anticipation should lead to an endeavour to prepare for the future. Refinements or developments apparent at the research stage should receive consideration so that sufficient evidence may be available to justify development studies. The question "Where do we go next?" is properly answered by the research programme.

Central Technical Administration

Closely planned activities, requiring prompt central administrative action, justify the existence of an Administration Group to act as liaison with the main Administrative, Finance, Engineering and Operations Branches. Under a technical head, this is mainly a technical group distinct from professional or financial administrators. They are in a position to have a better appreciation of the needs of the

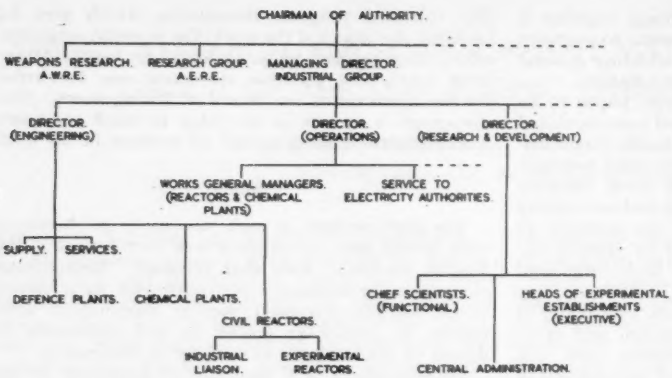


Fig. 1. Relationship of Research and Development Branch to activities requiring development effort

scientific staff and thus to act as a bridge between the main administration and the Branch.

The group collates the records supplied by all laboratories to form a master record of all effort in the Branch. These records of utilization of scientific and experimental effort are invaluable in the overall planning of work and in determining what commitments can be undertaken in the future.

The head of the Central Administration, as secretary of the Research Board, is responsible for the organizing of all business of the Research Board from the issuing of the agenda to the compilation of the minutes.

The Central Administration also has responsibility for all library and information services, although this does not extend further in the laboratories than ensuring uniformity of system. Much of the detail of methods used in the allocation and accounting of labour was worked out by the Central Administration.

Allocation of Responsibilities

The senior staff structure developed for the functional and executive control of applied research is shown in Fig. 1 in relation to the activities requiring development effort. Specific responsibilities carried by all members are integrated at the Research Board to result in agreed executive action. All bodies other than the Research Board are advisory, and all work arising from them must follow the formal Research Board sanction procedure. This is essential if firm control is to be maintained on the work of the experimental establishments. Fig. 2 shows the focal nature of the Research Board and the parts played by both functional and executive organizations in the representation of the Branch. Normally executive representation is required only for sanctioned commitments involving major effort.

The essential functional responsibility is to prepare a strategic plan linked to the overall scheme of development approved by the Board of Management, and then to co-ordinate all research and development effort according to that plan. Although tied to key dates for five to ten years, this must be sufficiently fluid to allow revision at, say, yearly intervals according to changing emphasis and the impact of experience. Promising researches must be related to an intimate knowledge of works practice and industrial development. Interchange of views and discussion on technical research problems must be arranged.

In general, sanctioned commitments always have the higher priority, but appreciable effort must be devoted to work in the earliest stages of feasibility and research studies. Such work suffers if done in isolation because the benefits conferred by expert consultants is lost. The findings of the work must be made known to all who can assist in assessing the directions in which they may be exploited. This is a functional responsibility, and staff with such duties should have direct access to the scientific workers at all levels in the laboratories so that the most up-to-date picture can always be presented. When the activity is large enough, it is convenient to divide the functional responsibilities into chemistry and chemical engineering, physics and engineering, and metallurgy, each under a senior member of the staff or chief scientist directly responsible to the Director of Research and Development.

The officer in charge of functional work should be of senior status, so that he has at least parity in discussion with chief engineers, works general managers, and heads of experimental establishments. Because of the difficulties in the comparative assessment of competing demands, a man of mature ability and experience able to take a detached view is essential for this work.

By collaboration with all parties who have an interest and can express a responsible opinion, the chief scientists advise on the forward forecast of research and development. Existing engineering commitments will have higher priority, and experi-

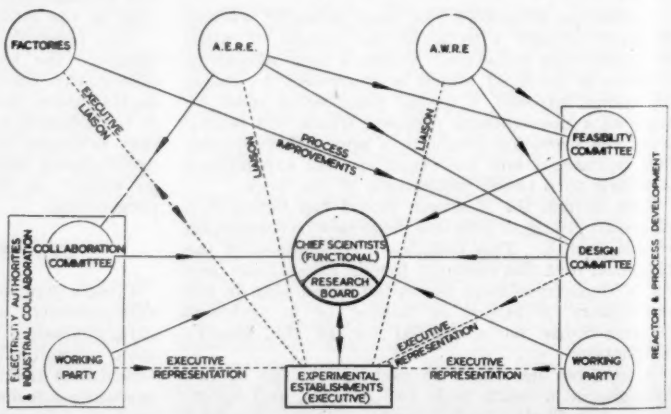


Fig. 2. Focal nature of Research Board

ence in the laboratories may suggest modifications. The several views are unified at the Research Board. After it is agreed, the strategic plan can be detailed, based on the accumulated records of work which should be compiled at the laboratories and from which the effort and cost of investigations similar in nature can be deduced.

The value of such a strategic plan is that it should show realistically how many development projects can be dealt with within existing strength, when key decisions are necessary related to overall construction or production plans, the expected utilization of labour and the possible need for recruitment. After formal executive approval, it should be distributed to senior members of other branches to apprise them of the extent of effort applied on their behalf and to allow the opportunity for further discussion on overall policy. Executively, it provides the key to effective utilization of effort.

Where a number of experimental establishments is involved, an important functional responsibility is to ensure that all work is co-ordinated according to the agreed plan, and all but necessary duplication avoided. Constant discussion with the experimental workers should mean that new findings are applied expeditiously even before publication.

Where facilities to cater for some aspect do not exist anywhere in the organization, arrangement for extra-mural research to be carried out by some other organization is appropriate to functional responsibility, although the management of such work when initiated is executive.

During the preparation of the budget submissions, the co-ordination of financial requirements can be made a functional responsibility, and thus the various schemes from the separate establishments can be dovetailed before consideration by the Research Board. This is a logical consequence of strategic planning.

Where the organization is large, strategic planning will embrace all aspects in all establishments or groups. Tactical planning applies only to the establishment, group or section the effort of which is under consideration, and is necessary to the efficient discharge of the executive responsibility of management.

INTERNATIONAL PROTECTION OF BIRDS

THE sixth Conference of the European Continental Section of the International Committee for Bird Preservation was held in the village of Beetsterzwaag, Friesland, Holland, during June 3–7, under the chairmanship of Dr. Boje Benzon (Denmark). The meeting was attended by delegates of the national sections of Austria, Belgium, Denmark, France, Germany, Great Britain and Northern Ireland, Italy, Netherlands, Norway, Sweden and Switzerland, together with representatives of the International Union of Biological Sciences, the Conseil International de la Chasse and an observer from Luxembourg.

One of the most important achievements of the European Section was the drawing up of an International Convention for the protection of birds which was signed *ad referendum* by the representatives of twelve European powers in Paris in 1950. Switzerland was the first country to ratify it in 1954, and was followed by the Netherlands, Spain, Belgium and Iceland; but in spite of the new Bird Protection Act of 1954 on which great hopes were placed, the United Kingdom Government has still expressed inability to ratify. This Convention is a revision of the Paris Convention of 1902, which was confined to birds useful to agriculture only, and embraces the modern conception of bird protection—that as a main principle all birds should be protected—exceptions being made for species which are considered harmful, and for special circumstances and conditions. After a frank discussion, the general feeling of the meeting was expressed in a resolution pointing out that certain governments might be hesitant to ratify the Convention on account of minor differences, and directing attention to the 'escape clauses' provided.

In another international agreement, vitally affecting birds, the International Convention for the Prevention of the Pollution of the Sea by Oil, the United Kingdom, however, has taken the lead, for it was at the Inter-Governmental Conference convened in London in 1954 that this Convention was drawn up. The United Kingdom was the first country to ratify it, in 1955; the representatives of Denmark, the German Federal Republic and Sweden reported that their Governments had since taken this step, while others stated that their respective Governments were in the process of passing, or on the point of introducing, legislation to enable ratification. Thus after more than thirty-five years, during which thousands of sea-birds have perished annually, a concrete step forward to combat this evil has been made.

The diminution of the birds of prey all over Europe is causing concern, and it was unanimously agreed that these birds were in need of better protection and that to achieve this end education as to their value was urgently required. In this matter Great Britain, where full protection is accorded to all birds of prey except the sparrowhawk (*Accipiter nisus*), is outdistanced only by The Netherlands, where all birds of prey, without exception, are protected. This overall protection was recently given in Holland as a result of the frequent mistakes which had been made in shooting other species which are difficult to distinguish from the sparrowhawk.

Reports on the diminution of the white stork were given from several countries, though from Austria and Hungary came news of an increase in numbers. The causes of the diminution of this large white and black bird are varied; as with most species, more intensive cultivation being considered one of the prime reasons. It was stated that large numbers of storks meet their death by flying into overhead wires and tall chimneys, and France and Spain were blamed for the fact that though the stork is legally protected in those countries, the law is not enforced and large numbers are shot. It was alleged that in Spain hunters especially aim at killing ringed storks as they like to collect the rings as trophies, and in view of this it has been decided in the Netherlands to cease all ringing of young storks for the present. A French speaker stated that, from his personal observations, white storks certainly did not appear to be diminishing in Central Africa, and put forward the suggestion that numbers of this species might possibly be losing their habit of northerly migration. In Holland and other countries suitable platforms have been erected to encourage storks to nest, and this scheme has already met with encouraging success; it would appear, however, that the birds are becoming lazy,

as it has been observed that they prefer nests already prepared to a mere nesting platform.

The use of pesticides and their effect on wild life was dealt with at length by speakers from The Netherlands and Germany and supported by statements from Belgium, France, Great Britain, Sweden and Switzerland. The great difficulties of this problem were fully realized and it was agreed that in view of the time required fully to assess the effects of pesticides on wild life and the constantly changing situation with regard to the production of these substances, this subject should be kept permanently on the agenda of the Committee. In the meantime, a resolution was passed urging all Governments to take the necessary steps to prohibit the further use or sale of any pesticide which had been proved to be excessively destructive to birds and other forms of wild life.

Among other subjects discussed at the meeting were the control of the export and import of wild birds' eggs, birds which are a menace to other species, the protection of the skylark and the effect of shooting during severe frost. National representatives also reported on the establishment of new bird reserves, particularly on migration routes, and the state of bird preservation in their respective countries.

Throughout the meeting it was evident how difficult it is for one country to pass and maintain protective measures unless her neighbours do likewise, and that, to ensure the effective preservation of birds, international collaboration is imperative. But the cordial atmosphere which prevailed, and the obvious desire of all national representatives, without exception, to co-operate to the fullest extent, were striking features of the discussions.

Immediately before the Conference, a meeting of the Executive Board of the International Wildfowl Research Bureau (a branch of the International Committee for Bird Preservation) was held under the chairmanship of the honorary director, Dr. Edward Hindle, at which the problems of the conservation of wildfowl, the Anatidae in particular, were dealt with.

Phyllis Barclay-Smith

INTERNATIONAL SCIENTIFIC RADIO UNION

An account of the eleventh general assembly of the International Scientific Radio Union, held in The Hague during August 23–September 2, 1954, has already appeared in *Nature* (September 3, 1955, p. 451); and this, together with a later account (*Nature*, April 7, p. 652), dealt with three (Parts 1, 4 and 8) of the eight parts of Vol. 10, which forms the published proceedings of the assembly. The remaining five parts have now been published*.

Part 2 deals with the work of Commission II on "Radio and Troposphere". As the chairman's summary of the proceedings shows, this Commission is very active in research on the influence of meteorological conditions on the propagation of radio waves by normal refraction processes to distances well beyond the horizon, and by the phenomena of scattering to distances of several hundred kilometres.

"Ionospheric Radio", the title of Commission III, is covered in Part 3. During the general assembly, this Commission held six technical sessions which dealt with the behaviour of the ionosphere at high latitudes, the morphology of the *D*-layer, rocket investigations of the ionosphere, absorption and forward scattering by the ionosphere, and with geomagnetic distortion and storms in the *F*2-layer. Many of the resolutions of the Commission are concerned with the important and very active part to be played by the International Union in the programme for the forthcoming International Geophysical Year.

The proceedings of the deliberations by Commission V, "Radio-Astronomy", are described in Part 5. The work of this Commission has already resulted in the publication of three special reports (see *Nature*, December 10, 1955, p. 1110), and the whole subject is being actively pursued on a basis of international co-operation. Sub-commissions have been established to study continuous measurements of solar radio emission, and to explore the possibilities of standardizing equipment and measuring techniques. The importance of making adequate frequency allocations for radio astronomy is emphasized; and in the case of such frequency bands as those in which the neutral hydrogen line is located, there is a need for international agreement on such allocations.

Part 6 describes the work carried out at the general assembly by Commission VI on "Radio Waves and Circuits". The resolutions of this Commission show that its work ranges over a wide field, including information and its measurement, the spectra of random signals, band-widths and time-constants, and the applications of abstract algebra to new circuit arrangements for equipment and antenna systems.

The title of Commission VII is "Radioelectronics", and the report of the chairman given at the end of Part 7 shows that there has been much discussion as to the manner in which the Commission will fit into the general pattern of the International Union. During the meetings, discussions were held on the subjects of solid-state and gaseous electronics, valves for microwave optics and electronics, and on the generation of radiation by gaseous processes; the last being dealt with jointly with Commission V as a subject of mutual interest. A recommendation was made to form a joint commission between the International Scientific Radio Union and the International Union of Pure and Applied Physics to provide means for the discussion of topics in electronics which do not properly belong in the field of radio.

All the publications referred to above contain full details of the scientific sessions, all the national reports in full, together with, in most cases, carefully selected lists of references; the summarizing reports of the respective chairmen with the resolutions of the Commissions adopted at the general assembly are also included. The publication of these remaining parts completes the official record of The Hague meeting of the International Scientific Radio Union, 1954.

* International Scientific Radio Union. Proceedings of the 11th General Assembly held in The Hague from August 23rd to September 2nd, 1954. Vol. 10, Part 2. Commission II: Radio and Troposphere. Pp. 90. 1956. 100 Belg. fr.; 14*s*. 6*d*.; 2 dollars. Vol. 10, Part 3. Commission III: Ionospheric Radio. Pp. 194. 1955. 200 Belg. fr.; 29*s*.; 4 dollars. Vol. 10, Part 5. Commission V: Radio-Astronomy. Pp. 114. 1955. 125 Belg. fr.; 18*s*.; 2.50 dollars. Vol. 10, Part 6. Commission VI: Radio Waves and Circuits. Pp. 140. 1955. 150 Belg. fr.; 21*s*. 6*d*.; 3 dollars. Vol. 10, Part 7. Commission VII: Radioelectronics. Pp. 140. 1956. 150 Belg. fr.; 21*s*. 6*d*.; 3 dollars. (International Scientific Radio Union, 42 rue des Minimes, Brussels.)

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY, CAMBRIDGE

REPORT FOR 1956

AT the annual general meeting of the Fellows of the National Institute of Agricultural Botany, held in Cambridge on July 13, Prof. E. T. Jones, director of the Welsh Plant Breeding Station, received the Institute's Cereal Award for his work in breeding the new winter oat 'Powys'. This variety combines the best features of the well-known *S.* 147 and *S.* 172 and has shown outstanding merit in a series of trials. It will not, however, be available for general distribution this year.

The thirty-sixth annual report* presented at the meeting records continued expansion of the work of the Institute and plans for further building-extensions have been under active consideration. Some changes in the organization of cereal trials have been completed, which will reduce the time before a decision regarding recommendation can be made from four to three years. 'Preliminary' trials of new varieties are now grown at four of the thirteen regional centres, and with the aid of special plot combine-harvesters reliable results can be obtained in the first year. The more promising varieties go into 'main' trials, with larger plots at more centres, incorporating three levels of nitrogenous top dressing, in addition to a basic dressing to ensure adequate supplies of phosphorus and potassium. Another new feature is the trial of varieties used as 'controls' during the past thirty-five years, to see how far modern cereals are improvements on the older ones. The transfer of responsibility for the production of stock seed of all the Aberystwyth strains of grasses and clovers from the Welsh Plant Breeding Station to the Institute has necessitated much reorganization of technical work and contracting arrangements, and a special granary extension for these seeds is in course of erection. Progress continues with the plans for supervising the quality of seed stocks grown in Britain. Cereal crops entered for inspection again exceeded 100,000 acres, and nearly 54,000 acres were field-approved. A Comprehensive Certification Scheme for special cereal seed stocks was operated on a limited scale for the first time in 1955, and it is hoped that the arrangements for the National Comprehensive Certification Scheme for Herbage Seed will be complete in 1956.

The report also provides useful information regarding the performance of different varieties of potato, pulses, roots, fodder and oil crops, and field vegetables. Management trials carried out with lucerne showed that the best time for the first cut is the early bud stage. This both gave the best-quality hay, and, as a result of the earlier second growth, enabled four cuts to be taken each season. For those interested in grasses, the classification of some seventy strains of perennial rye grass according to their time of flowering, and the results of trials with hormone weed-killers on grass seed crops are of particular value, relatively safe times for spraying being given for the different Aberystwyth strains.

Details of the work of the Official Seed Testing Station are included in the report. The large increase in cereal samples received for testing and the high percentage with germination which fell below the authorized minimum standard reflect the bad harvesting conditions in 1954.

* National Institute of Agricultural Botany. Thirty-sixth Report and Accounts, 1955. Pp. 54. (Cambridge: National Institute of Agricultural Botany, 1956.)

STUDIES IN MUTAGENESIS

Production of Bacteriophage Mutants by a Disturbance of Deoxyribonucleic Acid Metabolism

IN experiments in which the pyrimidine derivative 5-bromouracil was incorporated into the deoxyribonucleic acid of *T2r*$^+$ bacteriophage, according to the method of Dunn and Smith[1], it was observed that a large number of a variety of plaque-type mutants appeared among the progeny phages, as illustrated in Fig. 1. The observation of this apparently powerful mutagenic effect of 5-bromouracil led us to an investigation of the conditions upon which the production of phage mutant depends.

In a typical experiment, a culture of *Escherichia coli*, strain *B*, was grown with aeration at 37° C. in complete medium supplemented with 50 μgm./ml. 5-bromouracil, for four generations to a density of 2×10^8 cells/ml. (Complete medium is a glucose-salts medium[2] supplemented with 1 mgm./ml. sulphanilamide, 1 mgm./ml. vitamin-free casein hydrolysate, 25 μgm./ml. xanthine, and uracil equal to 5 per cent of any 5-bromouracil added. We had found that when commercial 5-bromouracil, which contained a 5 per cent uracil contamination, was added to complete medium, no further pyrimidines were necessary for bacterial growth. In complete medium plus pure 5-bromouracil, however, the bacteria required uracil for growth.) The bacteria were then centrifuged and resuspended in the same volume of complete medium plus various concentrations of 5-bromouracil, infected with 3×10^8 *T2r*$_2$ phages per ml., and the infection was allowed to proceed for 3 hr. At this time the bacteria were centrifuged, and the supernatant fluids were diluted and plated on broth agar. The results are presented in Fig. 2, where the 'infective yield' (the ratio of the number of infective, that is, plaque-forming, progeny to the number of input phage) is plotted as a function of the 5-bromouracil concentration in the medium. The percentage mutants (100 times the ratio of all mutant plaques to the total number of progeny plaques) is seen to be maximal at the concentration of 5-bromouracil giving minimal infective yield. No mutants were produced in the absence of 5-bromouracil. The quantitative results were observed to vary somewhat, depending on the exact growth conditions employed.

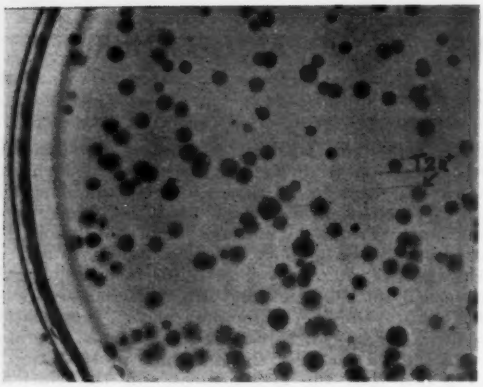


Fig. 1. Infective progeny of $T2r^+$ resulting from growth in complete medium plus 5-bromouracil

We have also studied the effects of other uracil derivatives and have found that 5-chlorouracil and 5-iodouracil, both of which are incorporated into phage deoxyribonucleic acid[1], gave yield and mutation patterns similar to those of 5-bromouracil, but that neither 5-aminouracil nor uracil itself produced mutants. In the absence of sulphanilamide, none of the three halogenated uracil derivatives had any mutagenic effect. Only plaque-type mutants have so far been investigated, but these have at times represented as high as 15 per cent of the total infective progeny. A large number of these mutants have been picked and replated three or more times on broth agar, and in almost all cases they kept their mutant identity. These mutants must, therefore, be stable and do not need 5-bromouracil to reproduce.

The appearance of mutants during growth in the presence of 5-bromouracil and sulphanilamide could be attributed either to a mutagenic action or to a selective enrichment of the mutants already present in the original stock at a frequency of 10^{-3}. The latter possibility was eliminated by the following two experiments. First, a one-step growth experiment[3] was performed. *E. coli* B was grown in complete medium plus 5-bromouracil as previously described and then infected in the same medium with one $T2r_3$ phage for every ten bacteria. After phage adsorption, but before lysis, the infection mixture was diluted into first and second growth-tubes containing 10^3 and 10 original phages per ml., respectively, and aliquots from each of the two tubes were plated at intervals before and after lysis. We found that mutant production was optimal when the phages were grown in the presence of at least 10^8 cells/ml. In order to simulate mass culture conditions in this experiment, the dilutions were made into a spent medium, that is, complete medium plus 50 μgm./ml. 5-bromouracil in which *E. coli* B had grown for 3 hr. and from which the bacteria had been afterwards removed by filtration. After lysis we found that while the total number of phages had increased some eighteenfold, the number of mutant phages had increased five hundredfold. To explain this result as due to selection, one would have to postulate a burst of five hundred for each pre-existing mutant. In this medium, however, the mutants were shown to have bursts of the same magnitude as the original stock. The second type of experiment was a single-cell burst experiment[4]. *E. coli* B was again grown as previously described, but this time infected with more than one $T2r_3$ phage per bacterium. After a few minutes, the unadsorbed phages were inactivated by dilution into *T2* antiserum, and the infected bacteria were further diluted down to a level of about 10 cells/ml. Single drops of this dilution were distributed into each of sixty-six tubes, and after allowing ample time for lysis to occur, each tube was plated separately.

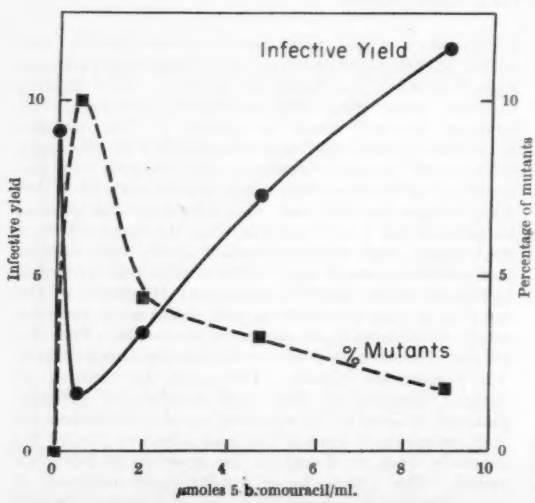


Fig. 2. Pattern of action of complete medium plus 5-bromouracil on phage production and mutation

In this experiment mass culture conditions were maintained by making the dilutions into a culture of 10^8 cells/ml. of uninfected *T2*-resistant *E. coli*, strain *B/2* growing in complete medium plus 5-bromouracil. The results are given in Table 1. Any pre-existing mutant should have developed into a clone of mutant progeny. This was not observed; rather, of the thirteen plates containing mutant plaques, twelve had one mutant per plate and one had two mutants. The quantity A is calculated from the number of bacteria originally infected with phage. B is obtained from the Poisson formula, $P(O) = e^{-n}$, where $P(O)$ is the proportion of plates containing no plaques, and n is the number of infected bacteria per plate. The ratio of B to A, the fraction of the infected bacteria which released phage, is equal to 1, and therefore every infected bacterium produced infective phage. C is calculated from the number of plates which contain no mutant plaques, again using the Poisson formula. The ratio of C to B is the fraction of infected bacteria which released mutant phage. Since in the original infection mixture only one out of every 10^3 bacteria was infected with a mutant phage, the mutants appearing after lysis from 1 in every 4 bacteria must have arisen through mutation and not selection.

Table 1. Single-cell Burst Experiment

No. of plates	No. of plaques/plate	No. plates with mutants
28	0	—
11	1–5	2
13	6–9	4*
9	10–21	4
5	22–85	3

A, infected cells/plate (calculated from input)	0·75
B, infected cells/plate (calculated from Poisson law)	0·86
Ratio of B to A	1·1
C, infected cells/plate yielding mutants (calculated from Poisson law)	0·22
Ratio of C to B	0·26

* One plate contained 2 mutants

The mutagenic effect of 5-bromouracil in the presence of sulphanilamide must certainly be connected with a disturbance of deoxyribonucleic acid metabolism or structure. Another instance, in a different system, of mutagenesis resulting from an interference with deoxyribonucleic acid metabolism, more specifically thymine metabolism, is presented in the following communication by Coughlin and Adelberg. The most obvious proposal to explain the mutagenic action of 5-bromouracil is that the incorporation of this base into deoxyribonucleic acid may be directly responsible for the appearance of phage mutants. Both Dunn and Smith[1] and we have found that a large proportion of phages (total phages being estimated from optical density at 260 mμ) containing 5-bromouracil are non-infectious. The amount of thymine replaced by 5-bromouracil seemed to depend on the 5-bromouracil concentration in the medium. We have obtained a phage preparation the thymine of which was completely replaced by 5-bromouracil, but 9 per cent of the total phages still formed plaques of normal and mutant type. As yet, however, no correlation can be made between the amount of thymine replaced by 5-bromouracil and the percentage of mutants among the surviving infective progeny.

Sulphanilamide is known to inhibit methyl transfer by interfering with folic acid formation, consequently reducing the ability of the bacteria to synthesize thymine. But the combination of 5-bromouracil and sulphanilamide has further metabolic effects, not yet clearly defined, on reactions required for pyrimidine biosynthesis. Aside from its incorporation into deoxyribonucleic acid, 5-bromouracil inhibits thymine utilization, is reversed by thymidine competitively, and inhibits uracil formation or utilization.

While at present no definitive mechanism for the mode of action of 5-bromouracil and sulphanilamide can be proposed, it is hoped that further investigations of the biochemical aspects of this system may lead us to a better understanding of the biochemical nature of mutagenesis.

We are grateful to Drs. G. S. Stent, J. D. Smith, E. A. Adelberg, and W. M. Stanley for valuable discussions and suggestions, and to Mr. J. Toby for taking the photograph.

This work was aided by grants from the Lederle Laboratories and the Rockefeller Foundation.

Rose M. Litman*
Arthur B. Pardee

Virus Laboratory,
University of California,
Berkeley, California.

* Abraham Rosenberg Fellow.

[1] Dunn, D. B., and Smith, J. D., *Nature*, **174**, 305 (1954).
[2] Herriott, R. M., and Barlow, J. L., *J. Gen. Physiol.*, **36**, 17 (1952).
[3] Adams, M. H., in "Methods in Medical Research" (ed. J. H. Comroe, jun.), **2**, 25 (Yearbook Publishers, Inc., Chicago, 1950).
[4] *Ibid.*, p. 35.

Bacterial Mutation induced by Thymine Starvation

The phenomenon of 'thymineless death' of *Escherichia coli* strain 15*T*⁻, a thymine-requiring mutant, was discovered by Cohen and Barner[1]. Their experiments demonstrated that these bacteria lose their ability to give rise to colonies following incubation in a thymine-free synthetic medium which supports rapid growth of the prototrophic parent strain *E. coli* 15.

The purpose of this report is to present experiments which indicate that thymine starvation of these organisms has a mutagenic effect on the surviving cells. The effect of thymine starvation on the appearance of new mutants has been studied in the following manner.

The parent strain employed, *E. coli* 15 *T*⁻*H*⁻*N*ˢ, requires both thymine and histidine for growth and is unable to grow in the presence of 100 μgm. of *nor*leucine per ml. This strain was kindly supplied by Miss Hazel Barner, who isolated it following ultra-violet irradiation of *E. coli* 15*T*⁻. The mutational event chosen for the study of mutagenesis was the one which conferred independence of histidine on the parent strain. An exponentially growing culture was placed under conditions of thymine starvation by washing it twice with distilled water and resuspending it in a 'basic mineral medium'[2] containing 2 mgm. of glucose and 10 μgm. of histidine per ml. At various times, aliquots were withdrawn from the thymine-free medium, chilled and divided into two samples. One sample was plated on broth agar for determinations of viable cell counts. The other sample was washed twice in distilled water and concentrated by centrifugation, then shaken for twenty minutes to free the suspension from clumps of cells. Aliquots of 0·1 ml. of the concentrated cell suspension containing about 1×10^8 cells/ml. were then spread on the surface of agar plates of 'basic mineral medium' containing 2 mgm. of glucose and 2 μgm. of thymine per ml. of washed agar. Colony counts of the presumptive histidine-independent mutants were made after incubation at 37° C. for five days, and random colonies were picked and tested to verify their thymine-dependent and histidine-independent conditions.

The ability of this procedure to recover histidine-independent (H^+) mutants was checked in the following manner. A suspension was prepared containing a known number of H^+ cells, and aliquots of this suspension were mixed with suspensions of histidine-dependent (H^-) cells in various ratios approximating actual experimental conditions. The individual suspensions and the mixtures were washed, concentrated and plated as described above; the results indicated that these procedures afford quantitative recoveries of mutant cells present in predominantly wild-type populations.

The results of a typical experiment are presented in Table 1, where it can be seen that a hundred-fold proportional increase of H^+ mutants appeared among the survivors of 185 min. of thymine starvation. This can be compared with the results of an ultra-violet irradiation of the same parent strain, which yielded a two-fold proportional increase of H^+ mutants among the survivors of a dose killing 98·6 per cent of the cells.

The results in Table 1, however, can be taken as only presumptive evidence of a mutagenic effect of

Table 1. Proportional Increase in H^+ Mutants during Thymine Starvation

$5{\cdot}8 \times 10^7$ cells, genotype *T*⁻*H*⁻*N*ˢ, suspended in thymine-free synthetic medium at time zero. Conditions as described in text

Minutes thymine starvation	Per cent killed	H^+ mutants per 10^8 viable cells	H^+ mutants per ml.
0	0	22	13
80	56	42	10
140	87	101	8
185	99	2,540	10

thymine starvation, since the absolute number of viable mutants per ml. of suspension did not increase. This could mean that thymine starvation exerted only a selective effect: that is, the H^+ mutants initially present in the untreated population could have survived thymine starvation better than the population at large.

An experiment was therefore performed in which a pure culture of T^-H^+ cells was subjected to thymine starvation. The H^+ cells were found to 'die' at a rate comparable to the rate of 'thymineless death' of H^- bacteria. Nevertheless, the possibility still remained that H^+ cells might behave differently in a suspension of mixed H^+ and H^- cells, where interactions between the two cell types might significantly alter the survival of H^+ organisms. Consequently, a more direct experiment was designed to test the survival of the H^+ cells under conditions simulating those of the actual mutagenesis experiment. For this purpose a small number of H^+ cells was mixed at zero time with a suspension of H^- organisms. The two cell types were distinguished by the presence of genetic markers for *nor*leucine sensitivity (N^s) and *nor*leucine resistance (N^r), respectively. The $T^-H^-N^r$ strain was obtained by plating a washed exponential phase culture of $T^-H^-N^s$ cells directly on synthetic growth medium containing 100 μgm./ml. of *nor*leucine, while the $T^-H^+N^s$ strain was obtained by plating the survivors of 185 min. of thymine starvation on synthetic medium lacking histidine.

The proportion of spontaneous *nor*leucine-resistant mutants in the original suspension of $T^-H^+N^s$ cells was 103 per 10^8 viable cells. However, only 220 cells of the latter type were added per ml. of mixed suspension at the beginning of the experiment, so that the presence of any significant numbers of $T^-H^+N^r$ cells among them was deemed highly unlikely.

A summary of the results of this experiment is presented in Table 2. The surviving numbers of added $T^-H^+N^s$ organisms per ml. of original suspension at any given time during thymine starvation was calculated by subtracting the number of colonies formed on agar containing glucose, thymine and *n r*leucine, from the number of colonies arising on similar medium devoid of *nor*leucine. The validity of this method was verified by picking a random sample of H^+ mutants from the *nor*leucine and minimal plates and testing them for sensitivity to *nor*leucine as well as for histidine independence and thymine dependence. From the data presented in Table 2 it can be seen that all the $T^-H^+N^s$ cells added at the beginning of the experiment were eliminated from the population after 225 min. of thymine starvation, establishing that a selective mechanism favouring the preferential survival of histidine-independent cells is not operative during our experimental conditions. Hence the substantial increase in the proportion of $T^-H^+N^r$ mutants can best be explained as resulting from an induction of new mutants.

It may also be noted in Table 2 that while the $T^-H^-N^r$ cells undergo a 13 per cent reduction in viable cell count due to thymine starvation, the $T^-H^+N^r$ cells are reduced from 53 per ml. to 7 per ml.: a reduction of 87 per cent. This disproportionate death of H^+ mutants which have arisen spontaneously in a growing culture of H^- cells has been observed repeatedly in the early stages of thymineless death, and is probably a consequence of the multinucleate condition of the bacteria and the dominant nature of the H^+ condition[3]. If the loss of viability due to thymine starvation is the result of random nuclear inactivation, as indicated by the work of Fuerst and Stent[4], then a bacterium would die only after all its nuclei had been inactivated. An H^+ mutant, on the other hand, would effectively disappear from the population following inactivation of only those nuclei specifically carrying the H^+ mutant character. If a significant proportion of the H^+N^r bacteria were heterokaryotic with respect to the H^+ genotype, bacterial cell 'death' would not be equivalent to mutant or nuclear 'death'. Hence in the initial stages of 'thymineless death', where the majority of the bacteria are presumably in a multinucleate condition, the mutant (H^+) count would decrease at a faster rate than the viable count.

It should be noted that in the reported experiments no provision was made for the delayed expression of mutation, since the washed cells were plated directly on media devoid of histidine. Indeed, the results of one experiment in which the survivors were plated on limiting amounts of histidine showed that only a fraction of all the induced mutants is recovered with our standard procedure. The true number of induced mutants is, therefore, greater than that indicated by the figures in the tables.

Any explanation of the causative mechanism for the reported mutagenesis would be strictly speculative at present. It is of interest, however, that Dunn and Smith[5] have reported an increased appearance of the base 6-methylaminopurine in the deoxyribonucleic acid of strain 15T^- under conditions of limiting thymine, and the relation of the metabolism of this base to the mutagenic activity of thymineless death is now under investigation.

The experiments of Litman and Pardee (see previous communication) have indicated a correlation between appearance of coliphage mutants and interference with deoxyribonucleic acid metabolism. From our experiments it might also be inferred that bacterial mutation can be a consequence of disrupted deoxyribonucleic acid metabolism. For the moment, however, we can conclude only that the state of specific thymine starvation is mutagenic in *E. coli* for the one phenotypic property studied.

This work was supported by the Division of Biology and Medicine of the U.S. Atomic Energy Commission and by a grant from the National Science Foundation.

Table 2. Thymine Starvation of $T^-H^+N^s$ Cells in Mixed Suspension with $T^-H^-N^r$ Cells

Cells of type $T^-H^-N^r$ mixed with cells of type $T^-H^+N^s$ at time zero to give final cell densities of $6{\cdot}2 \times 10^7$ and 220 per ml., respectively. Details in text

Per cent H^-N^r cells killed	H^+N^r mutants per ml.	H^+N^r mutants per 10^8 viable cells	H^+N^s mutants per ml.
0	53	86	220
13	7	14	—
50	20	65	82
76	50	345	1
92	59	1,162	0

Carroll A. Coughlin
Edward A. Adelberg

Department of Bacteriology,
University of California,
Berkeley.

[1] Barner, H. D., and Cohen, S. S., *J. Bact.*, **68**, 80 (1954).
[2] Cohen, S. S., and Arbogast, R., *J. Exp. Med.*, **91**, 619 (1950).
[3] Ryan, F. J., *Proc. U.S. Nat. Acad. Sci.*, **40**, 178 (1954). Ryan, F. J., Fried, P., and Schwartz, M., *J. Gen. Microbiol.*, **11**, 380 (1954).
[4] Fuerst, C. R., and Stent, G. S., *J. Gen. Physiol.* (in the press).
[5] Dunn, D. B., and Smith, J. D., *Nature*, **175**, 336 (1955).

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

The Sun's General Magnetic Field

SOME of the recent theoretical discussions of the magnetic field of the Sun have tended to throw the subject into a state of confusion[1–3]. In particular, H. Alfvén[3] asserts that ". . . it is at present impossible to derive a value of the sun's general magnetic field from spectroscopic measurements", and that "quite without justification it is assumed that Zeeman-effect measurements give an accurate value of the solar magnetic field".

Such a statement is in conflict with the published observations of weak solar magnetic fields, and of the discussion that has been given to these observations[4].

Alfvén begins with the not unreasonable assumption that in the turbulent photosphere there is equipartition of energy between the kinetic and the magnetic modes. He argues that the intensity of the spectral line in which the Zeeman effect is measured is a function, f, of density, ρ, and temperature, T, and that in the granules ρ, T and the magnetic field H are coupled, so that "it is not at all likely that the average Zeeman effect gives an average of the magnetic field". Alfvén assumes (apparently on the basis of a particular theory of the origin of sunspots) that there is a general field H_0 of the order of 10 gauss. This is superimposed on the turbulent field H_t, so that in some parts of a granule the resulting field is $H_0 + H_t = +10 + 300 = +310$ gauss, whereas in other parts of it the field is $H_0 - H_t = +10 - 300 = -290$ gauss. The average then depends on the function $f(\rho, T)$ and the way in which ρ and T are coupled with H. If f is systematically smaller for positive values of H_t than for negative values, "the measured H_m may very well be negative even if the real field H is positive, and its absolute value may differ by orders of magnitude". Hence, according to Alfvén, "no conclusion can be drawn about the general magnetic field from the Zeeman-effect measurements and the results of these measurements are not relevant".

I believe that Alfvén greatly over-estimates the importance of the possible coupling of H with line intensity, and that his conclusion may readily be refuted by consideration of the published observations.

According to Alfvén's argument, the systematic effect of f and the way in which ρ and T are coupled with H should provide a large bias (probably subject to a centre-to-limb effect) underlying the much smaller true field. But the measurements of the Zeeman effect are absolute, and they show no trace of bias. Vast areas of the Sun, and occasionally, near sunspot minimum, the greater part of the solar disk, show no observable field distinguishable from zero. The limit is about 0·5 gauss for the 'noise' peaks, and much lower for any possible bias.

A further very significant result that was brought out in the discussion of the extensive series of observations with the solar magnetograph concerns the measured magnetic fields of bipolar magnetic (BM) regions, which exhibit a vast range of total magnetic flux. The large bipolar magnetic regions, while young, characteristically contain leading and following sunspots of opposite polarity. For such spots, G. E. Hale[5] established the well-known laws of magnetic polarity that pertain to the N- and S-solar hemispheres and to the alternation in successive 11-year sunspot cycles. As we have indicated, well-defined bipolar magnetic regions have approximate equality of positive and negative flux in the respective parts; the observations substantiate the concept of a localized loop of a submerged toroidal field rising above the surface, as suggested by Cowling[6]. Now in the N- or S-hemisphere of the Sun, in a given sunspot cycle, the same relative polarity of leading and following parts of all well-defined bipolar magnetic regions is exhibited over the whole range of magnetic intensity, from the very strong, spotted bipolar magnetic regions ($H \approx 3{,}000$ gauss) down to those (unspotted) of minimal size having average field intensities of only one or two gauss. Furthermore, weak bipolar and unipolar magnetic (UM) regions are frequently identifiable and measurable for days or weeks as they are carried across the disk by the solar rotation, even though they may be surrounded by vast areas having no outstanding field.

This disposes completely of the idea that the turbulent field of the granules systematically reverses, or appreciably biases, the measured Zeeman effects of weak magnetic regions on the Sun.

If, then, the polarity and approximate magnetic intensity of weak bipolar magnetic regions can be reliably observed, there is no reason for doubting the observations of weak fields, consistently positive near the north pole, negative near the south pole, that constitute the 'general' magnetic field of the Sun. After correction for darkening at the limb and for the presumed projection factor, the average intensity of the general field near the poles has been stated to be of the order of one gauss[4]. This is an order of magnitude weaker than the field required by Alfvén's theory of sunspots, and as Alfvén states, his solar model "seems to be in striking conflict with the Zeeman-effect measurements if these are interpreted in the naïve way as really measuring an average field". Since there is as yet no generally accepted theory of the magneto-hydrodynamic effects in solar granules, and since it would appear that such a theory, when finally perfected, will probably lead to rather minor refinements of the measured field intensities when averaged over large regions, we may have to continue with the present interpretation of the observations.

It may be mentioned that a second solar magnetograph[7], incorporating certain technical improvements and operating with the advantage of the better sky of Mount Wilson as compared to Pasadena, confirms the existence and polarity of the weak polar fields of the Sun previously reported.

HORACE W. BABCOCK

Mount Wilson and Palomar Observatories,
Pasadena, California.
June 13.

[1] Alfvén, H., *Arkiv för Fysik*, **4**, No. 24 (1952).
[2] Alfvén, H., *Nature*, **168**, 1036 (1936).
[3] Alfvén, H., *Tellus*, **8**, No. 1, 1 (1956).
[4] Babcock, H. W., and Babcock, H. D., *Astrophys. J.*, **121**, 349 (1955).
[5] Hale, G. E., *Astrophys. J.*, **49**, 153 (1919); *Nature*, **113**, 105 (1924).
[6] Cowling, T. G., "The Sun", 575, edit. by G. P. Kuiper (Univ. Chicago Press, 1953).
[7] Babcock, H. W., *Astrophys. J.*, **118**, 387 (1953).

Determination of Changes in the Dimensions of the Earth from Palæogeographical Data

Essentially, there are two opinions concerning the volume of the Earth: (1) its volume is decreasing, that is, the Earth is shrinking; (2) its volume has remained practically constant during geological times.

Geological observations have clearly established that the volume of the hydrosphere has grown throughout the geological past; the increase, however, has not been more than about 4 per cent of the whole volume since early Cambrian times.

In the case of a shrinking Earth, the average depth of the oceans must have increased. As a consequence of the higher sea-level, the area of the continents covered by sea water should be increasing.

If the volume of the Earth has remained constant, the amount of water-covered areas is influenced only by crustal movements and, on average, it should oscillate about a constant value.

The problem of the constancy or otherwise of the volume of the Earth can be solved, therefore, on the basis of palæogeographical data. We have only to establish the amount of the areas covered by sea water in different geological ages: a definite increase in the average values means a shrinking Earth, whereas their constancy indicates that on balance the volume of the Earth has not appreciably changed.

Fig. 1 represents the water-covered continental areas computed from the palæogeographical maps of T. M. Strahow; Fig. 2 is a similar diagram compiled from thirty-four palæogeographical maps recorded by H. Termier and G. Termier.

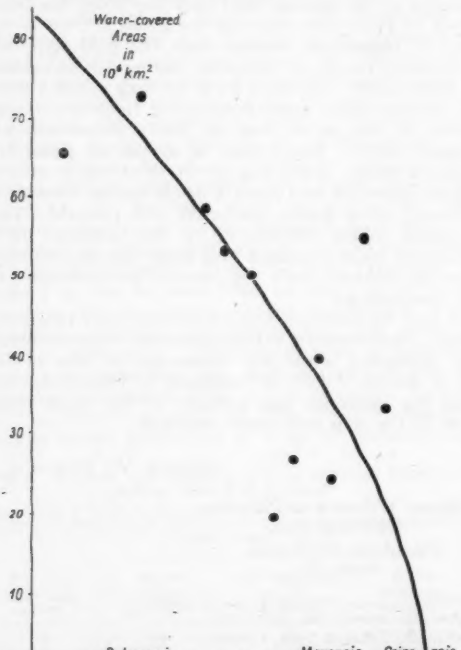


Fig. 1

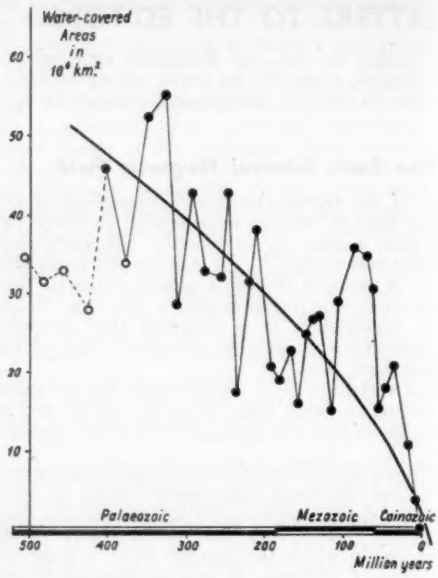


Fig. 2

In both diagrams the data plotted show a definite decrease in the course of the geological ages, indicating that there has been a cumulative increase of the radius of the Earth. Indeed, palæogeographical data provide evidence for the hypothesis that the Earth has expanded.

Figs. 1 and 2 can be used for giving the average rate of increase of the radius of the Earth, assuming that, on average, the hypsographic character of the Earth has remained the same since early Palæozoic times. Fig. 1 yields a radius increase of 0·66 mm./year; Fig. 2, compiled from the Termiers' data, gives 0·4 mm./year.

It is concluded that the annual increase of radius, according to palæogeographical data, has averaged about 0·5 mm./year during the past 500 million years.

L. Egyed

Geophysical Institute,
Eötvös L. University,
Budapest.

April 12.

Magnetic Perturbation of the Lowest Triplet States of Aromatic Molecules by Dissolved Oxygen

The absorption spectrum of benzene in the region 2795–3650 A. has been measured by Miss Pitts[1]. She observed a shoulder at approximately 2900 A. which has been tentatively assigned by Pariser[2] as a transition to a $^3E_{1u}$ state. Since it had been shown that oxygen dissolved in benzene absorbs strongly in this region[3], the spectrum has been re-investigated using pure benzene saturated with air or with nitrogen. A Beckman Model *DU* spectrophotometer with 5-cm. silica cells was used, and the results obtained are shown in Fig. 1. No shoulder around 2900 A. can be detected, although in most other respects the curve for air-saturated benzene is in reasonably good agreement with that of Miss Pitts. The absorption

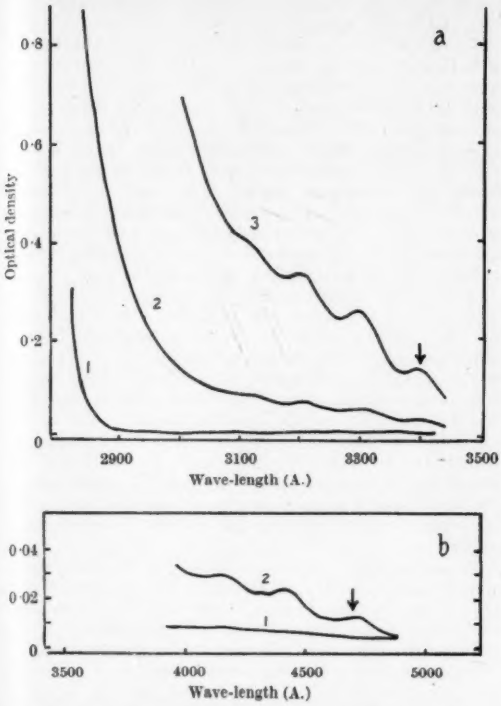


Fig. 1. (*a*) Absorption of liquid benzene saturated with (1) nitrogen; (2) air; (3) oxygen. (Blank—'Spectroscopic' methyl cyclohexane.)
(*b*) Absorption of naphthalene (2·8 *M* in ethylene dichloride) saturated with (1) nitrogen; (2) oxygen; and measured in a 5-cm. cell. (Blank: A solution of methyl cyclohexane in ethylene dichloride.)
All measurements at approximately 22° C. Arrows indicate the position of the shortest wave-length phosphorescence bands (ref. 4)

spectrum of the pure compound at room temperature therefore seems to provide no evidence for a triplet-level of benzene at about 34,000 cm.$^{-1}$.

A striking effect was, however, observed at longer wave-lengths. The well-established[1,4] 'triplet' bands around 3300 A. disappeared when the dissolved atmospheric oxygen was replaced by nitrogen. Conversely, they appeared much more strongly in benzene saturated with pure oxygen (see Fig. 1*a*). There is little doubt that these bands are in fact due to a transition to a triplet state (probably $^3B_{1u}$[2]). Thus they show the usual approximate 'mirror image' relationship with the phosphorescence bands[4]. (The absorption band at 3507 A. reported by Miss Pitts had not been observed previously, and was not found in the present work, even when 10-cm. cells were used.) The effect of the dissolved oxygen may accordingly be attributed to spin-orbit perturbation of the triplet-level by the inhomogeneous field of the paramagnetic oxygen molecule. (Oxygen dissolved in benzene has its normal paramagnetic susceptibility[5].) That magnetic perturbation of higher singlet–triplet transitions might be responsible for the principal absorption shown by oxygen dissolved in aromatic solvents was originally considered by Kasha (personal communication). A magnetic perturbation of a singlet–triplet transition has previously been observed in emission by Yuster and Weissman[6], who found that the phosphorescence life-time of the gadolinium complex of dibenzoyl methane was about 1/50 of that expected from the life-times of the lanthanum and lutetium complexes. Since a saturated solution of oxygen in benzene is approximately 11 *M* with respect to benzene, and only 0·008 *M* with respect to oxygen, the perturbing effect of the oxygen molecule is very much greater than that of the gadolinium ion, in spite of its smaller magnetic moment. Weak (possibly collisional type) complex formation probably takes place between benzene and oxygen, but it is unlikely that this alone will appreciably increase the singlet–triplet probability.

It is clear that the unperturbed singlet–triplet bands of benzene must be considerably weaker than has been previously supposed. This is supported by the long phosphorescence life-time of the benzene triplet[4].

Similar effects have also been observed for naphthalene, where the singlet–triplet bands in absorption were located for the first time (Fig. 1*b*). Magnetic perturbation by oxygen (possibly under pressure) may therefore, in certain cases, be a useful alternative to the heavy-atom technique[4] for detecting and confirming triplet-levels of aromatic molecules. The triplet-level of bromobenzene is already considerably perturbed by the heavy bromine atom, and very little change in the well-defined triplet absorption band was observed on saturation with oxygen.

Finally, it may be noted that the above results support the suggestion[7] that the quenching effect of oxygen (and nitric oxide) on the fluorescence of aromatic hydrocarbons is due to a magnetic perturbation which facilitates the excited singlet–triplet radiationless transition.

I wish to thank the University of Oxford for an I.C.I. Research Fellowship.

D. F. Evans*

Inorganic Chemistry Laboratory,
Oxford. May 8.

* Present address: Department of Chemistry, Imperial College of Science and Technology, London, S.W.7.

[1] Pitts, A. C., *J. Chem. Phys.*, **18**, 1416 (1950).
[2] Parlser, R., *J. Chem. Phys.*, **24**, 250 (1956).
[3] Evans, D. F., *J. Chem. Soc.*, 345 (1953).
[4] West, W., edit., "Technique of Organic Chemistry", **9**, 740 (Interscl. Pub., 1956).
[5] Eggleston, B. C., Evans, D. F., and Richards, R. E., *J. Chem. Soc.*, 941 (1954).
[6] Yuster, P., and Weissman, S. I., *J. Chem. Phys.*, **17**, 1182 (1949).
[7] Stevens, B., *Trans. Farad. Soc.*, **51**, 611 (1955).

Pitch of Inharmonic Signals

It is well established that a sound consisting of adjacent harmonics is perceived as having the pitch of the fundamental frequency. This pitch is to be attributed to a component of subjective perception, that is, the combined impression of some or all of the tones presented. Such a component, which is not susceptible to further aural resolution, is called a residue[1].

This phenomenon has been studied by the use of an amplitude-modulation method which permits the production of inharmonic signals as well. A pure-tone carrier A_x is modulated by a signal consisting of N components with frequencies B_x, $2B_x$, etc. The modulator produces a signal containing the carrier and the upper and lower side-bands. The components are equidistant but not necessarily harmonic.

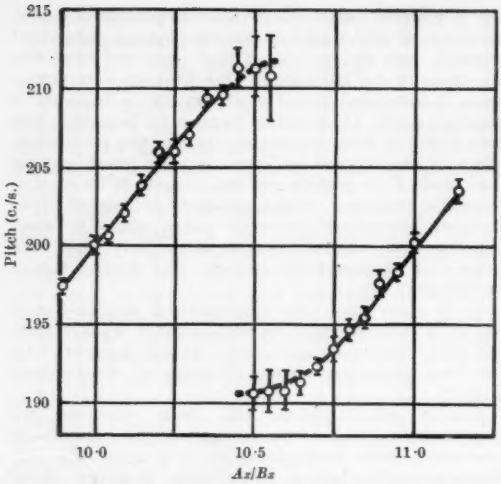


Fig. 1. Pitch versus carrier frequency. Modulation frequency 200 c./s.

It is observed that such 'coherent' inharmonic signals sound as stable and smooth as harmonic signals. The pitch has been determined by alternating the signal (X) with a harmonic signal (S) of adjustable fundamental frequency. The comparison signal (S) is produced by the same method as (X), the modulation frequency B_s being synchronized by the carrier A_s. The listener is then asked to vary the frequency A_s (which causes a corresponding variation of B_s) until the sounds (X) and (S) have the same pitch. The pitch-level of (X) is then the frequency B_s.

A typical result showing the dependence of pitch on the carrier frequency is shown in Fig. 1. In this experiment B_x has been kept constant. From Fig. 1 it will be noticed that the pitch increases with increasing carrier frequency. The relative variations of pitch in the neighbourhood of a harmonic situation are slightly larger than the relative variations of the carrier frequency. Midway between two harmonic situations, the pitch is indeterminate. The point exactly half-way is characterized by the component frequencies being odd multiples of half the modulation frequency. Careful listening reveals no trace of a pitch corresponding to $\frac{1}{2}B_x$, notwithstanding the low tonality of the sound.

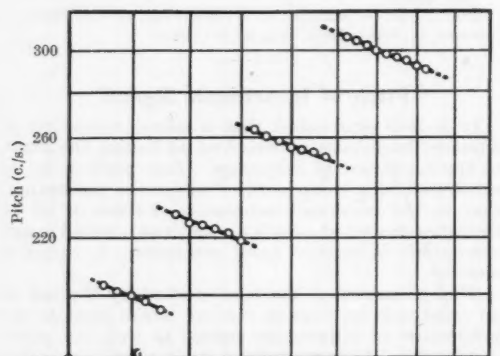


Fig. 2. Pitch versus modulation frequency. Carrier frequency 1,800 c./s.

When the pitch in the neighbourhood of a harmonic situation is exactly proportional to the carrier frequency, the pitch should be independent of the modulation frequency. The deviations from proportionality were therefore isolated in a second experiment in which the modulation frequency is varied. Fig. 2 shows a typical result of an experiment with constant carrier. Around a harmonic situation, the pitch drops with increasing modulation frequency B_x. The graph is again composed of several branches that are centred around harmonic situations and separated by regions of doubt.

A coherent inharmonic signal repeats itself over a much longer time than the period of the modulation signal. In the experiments, no beats due to the inharmonicity are observed, as long as the components of the stimulus are perfectly equidistant. This means that, when the envelope of the wave-form is constant, the tone quality of the residue is not affected by variation of phase or frequency of the fast oscillations within the envelope (which are determined by the carrier). In experiments that involve a change of the envelope, for example, by using an incoherent modulation signal, the residue quality is found to be affected[2].

It seems that the tone quality of the residue is greatly determined by the envelope of the signal, and the pitch by the 'fine-structure'. This simplified separation of phase and pitch effects is manifest in nearly all cases, except when the signal approaches the frequency-modulation condition[3].

E. de Boer

Ear, Nose and Throat Clinic,
Wilhelmina Hospital,
Amsterdam.
April 3.

[1] Schouten, J. F., *Proc. Acad. Sci. Amsterdam*, **43**, 356 (1940); *Philips Tech. Rev.*, **5**, 286 (1940).
[2] Licklider, J. C. R., *J. Acoust. Soc. Amer.*, **27**, 996 (1955); Chap. 25 of S. S. Stevens's "Handbook of Experimental Psychology", see p. 1019 (Wiley, New York).
[3] Boer, E. de, "On the 'Residue' in Hearing", thesis, Amsterdam (1956).

Cleavage Fracture of Bismuth Single Crystals

Earlier work[1] on the tensile stress necessary to cause cleavage fracture in zinc single crystals has shown that Sohncke's law was not obeyed. (Sohncke's law states that a crystal will break when the stress normal to the cleavage plane reaches some critical value). However, it is clear from the literature[2] that for some metal single crystals, notably bismuth, antimony and tellurium, Sohncke's law is obeyed. A study of the cleavage fracture of bismuth crystals was therefore undertaken in an attempt to discover the underlying cause of the difference of behaviour of zinc and bismuth.

The bismuth used contained 0·008 per cent lead; 0·0046 per cent tin; 0·0040 per cent iron; 0·0024 per cent antimony; 0·0018 per cent arsenic; 0·0015 per cent copper; 0·0015 per cent sulphur. It proved impossible to extract single crystals grown in precision-bore tubes without causing mechanical twins, and a technique for growing single crystals from a metal rod was developed from a method described by Noggle[3]. A moist mixture of alumina with an alumina clay bond was rammed around a poly-

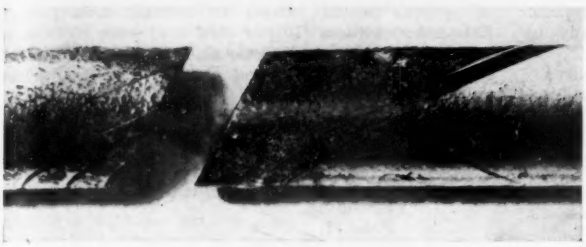


Fig. 1. Fractured bismuth crystal, illustrating crystallographic difference between cleavage and composition planes. × 3

crystalline rod to form the mould and, after drying, the single crystal was then grown. The mould material powdered very easily and the single crystal could be extracted without damage. The crystals used were about 7 mm. in diameter and at least 60 mm. long.

The crystals were orientated by the Laue back-reflexion method. The rhombohedral lattice of bismuth is very nearly face-centred cubic, and high accuracy of angular measurements is necessary to distinguish a (111) plane with certainty from a (11$\bar{1}$). (Both types of planes are possible cleavage planes.) The necessary angles and method used are to be published elsewhere. As yet, it has not proved possible to grow crystals of all orientations, and tensile tests have so far only been carried out on crystals with χ_{111} (the angle between the (111) plane and the longitudinal axis of the crystal) between 0° and 35°. Before the tensile test, all crystals were first etched and examined carefully for any signs of twins; generally none was visible. They were then electro-polished. The tensile tests were carried out at room temperature, the load being applied as axially as possible. In two of the crystals there were signs of twins developing as the load was increased to fracture; the behaviour of the other crystals gave no cause for suspecting the development of twins during the increase of load. But all crystals were etched after fracture so that the position of any twins could be observed.

This etch revealed a totally unexpected feature which apparently has not been noted in the earlier work on bismuth single crystals. The cleavage fracture did not, as in zinc, separate two parts of the specimen with the same lattice orientation, but was a plane separating a large twin from the original matrix. Fig. 1 shows clearly that on one side of the fracture there is a large twin extending completely across the crystal diameter. The other side of the fracture is almost entirely the original matrix, although there are one or two small twin lamellæ.

The crystallographic nature of the planes of the cleavage has been established as the (11$\bar{1}$) plane in the matrix and (111) plane in the twin. This is not the same as the normal composition plane of the twin and matrix, the (110). The difference between the two planes is obvious in Fig. 1. Thus, while the phenomenon resembles the 'parting' phenomenon in calcite[4], there is a difference in that the latter occurs at the twin/matrix composition plane. Zappfe[5] has seen the development of cracks between twins and matrix in bismuth–antimony alloys, which he describes as 'parting' on the twin matrix composition plane. But since he was working on polycrystalline material, crystallographic identification was difficult and the phenomenon may have been the same as that described here. Cahn[6] has found evidence of the existence of 'parting' in uranium; but he also could not examine the matter in any detail because of experimental difficulties.

The tensile stresses necessary to cause fracture in the crystals have been measured. There is a considerable scatter in the results, probably associated with the small amount of plastic deformation before fracture, usually less than 6 per cent. However, it is clear that the resolved stress perpendicular to the cleavage plane is approximately constant for all the twenty-six crystals breaking between twin and matrix, the average value being 583 gm./mm.^{2}. This is somewhat lower than the value of 695 gm./mm.^{2} determined by Wassermann[7] for 4-mm. diameter crystals of similar orientations.

It was found that in all the above twenty-six crystals the resolved stress across the most favourably orientated twin plane and in the twin direction exceeded the critical value for the growth of twins, as determined by Gyndyn and Starsev[8]. Four crystals did not show this phenomenon of separation as described above, but broke by a true cleavage on either a (111) or a (11$\bar{1}$) plane. In three of these four crystals the applied stress was not great enough to cause a twin to grow. It was interesting to note that in these crystals also the resolved stress perpendicular to the cleavage plane was less than in the main group of crystals.

Our thanks are due to Messrs. Mining and Chemical Products, Ltd., for the gift of the bismuth used in this work.

W. Vickers
G. B. Greenough

Department of Metallurgy,
University of Sheffield.
June 30.

[1] Deruyttere, A., and Greenough, G. B., *J. Inst. Metals*, **84**, 337 (1956).
[2] Schmid, E., and Boas, W., "Kristallplastizitaet" (Springer, Berlin, 1935).
[3] Noggle, T. S., *Rev. Sci. Instr.*, **24**, 184 (1953).
[4] Miers, H. A., "Mineralogy" (Macmillan, London, 1929).
[5] Zappfe, C., *Z. Metallkunde*, **44**, 397 (1953).
[6] Cahn, R. W., *Acta Metall.*, **1**, 49 (1953).
[7] Wassermann, G., *Z. Krist.*, **75**, 369 (1930).
[8] Gyndyn, A., and Starsev, V. I., *Zh. Eksper. Teor. Fiz.*, **20**, 738 (1950).

Detection of Iodine-containing Substances on Paper Chromatograms

The most sensitive chemical method of detection of iodine-containing substances on paper chromatograms is the application of the ceric sulphate–arsenious acid reaction[1]. By the use of this method the iodine-containing substances are indicated by white spots on a yellow background. Fletcher and Stanley[2] obtained white spots on a brown background by spraying such chromatograms with 1 per cent *o*-phenylenediamine in acetone.

We added ferroine to the ceric sulphate–arsenious acid reagent to make up the final concentration of this compound to 0·01–0·0066 *M*. The technique of detection was the same as that described by Bowden *et al.*[1]. By using this method we have obtained red spots on a blue background. This

contrasting colour reaction is stable for approximately 48 hr., and may be registered simply by direct photography.

I. Dragúnová
P. Langer

Institute of Endocrinology,
Slovak Academy of Sciences,
Bratislava, Czechoslovakia.
April 19.

[1] Bowden, C. H., Maclagan, N. F., and Wilkinson, J. H., *Biochem. J.*, **59**, 93 (1955).
[2] Fletcher, K., and Stanley, P. G., *Nature*, **175**, 730 (1955).

Detection of Triterpenoid Glycosides on Paper Chromatograms

In the course of our investigations of *Echinocystis lobata* seeds, it became desirable to locate triterpenoid glycosides on paper chromatograms by means of a colour reaction. A modification of the Liebermann–Burchard test described by Neher and Wettstein[1] was unsatisfactory for this purpose. We found that the original Liebermann–Burchard reaction applied to paper chromatograms with comparatively little alterations gave more satisfactory results, permitting a reliable detection of the spots.

The procedure is as follows. The dried paper chromatogram is placed on a glass plate and sprayed with a mixture of equal volumes of chloroform and acetic acid anhydride. A thin layer of concentrated sulphuric acid is spread on a glass plate and the treated filter paper strip laid on it. Additional sulphuric acid is smeared on the top of the strip with a glass rod. After a few minutes, the triterpenoid glycosides develop red-coloured spots. The starting line and the front, as well as the spots, can be marked on the other side of the glass plate in order to obtain the R_F values. This has to be done before the paper is destroyed by the sulphuric acid. Using Whatman No. 1 paper, after 15 min. the destruction is so far completed as to prevent the precise measurement of distances. The sensitivity of the test can be increased by examination of the chromatogram in ultra-violet light. The spots show orange fluorescence. In Table 1 are collected results, observed with pure echinocystic acid glycoside, isolated from *Echinocystis lobata* seeds. Similar results are obtained with methanolic extracts from the seeds.

The presence of the usual plant constituents (for example, oligosaccharides and amino-acids) does not interfere with the reaction. This method has been applied to the detection of triterpenoid compounds of several plants known to contain triterpenes[2]. Ethanolic extracts (70 per cent v/v) were chromatographed with butanol/acetic acid[2] and the chromatograms treated as described above. The results are shown in Table 2.

Table 1. Paper Chromatography of Echinocystic Acid Glycoside

Solvent	R_F	Sensitivity in visible light	Sensitivity in ultra-violet light	Paper
Butanol/acetic acid according to Partridge (ref. 2); descending	0·36	10γ	1γ	Whatman No. 1
Ethyl acetate with 0·08 addition of 1·5 per cent acetic acid and 2 per cent methanol, saturated with water until slight cloudiness appears; ascending	0·08	10γ	1γ	Whatman No. 1

Table 2. Paper Chromatography of Extracts from Triterpene-containing Plants

Plants	Solvent	R_F of glycoside	Paper
Callendula off.	Butanol/acetic acid according to Partridge (ref. 2)	0·8	Whatman No. 1
Arnica montana		no definite spot*	
Tussilago farfara		0·18 and 0·65	

* A coloured trail along the strip from the start to the front.

I. Belič

Chemical Department,
Medical Faculty,
University of Ljubljana,
Yugoslavia.
Feb. 11.

[1] Neher, R., and Wettstein, A., *Helv. Chim. Acta*, **34**, 2283 (1951).
[2] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
[3] Brieskorn, C. H., *Planta Medica*, **2**, 35 (1954).

Separation of Pyridine Mono-Carboxylic Acids on Paper Chromatograms

Chemical methods using cyanogen bromide and aromatic amines[1–3] (König reaction) and an acid-indicator[4] for the detection of pyridine carboxylic acids on paper chromatograms have been reported. But the toxic character of cyanogen bromide causes difficulty; and it has been observed that good spots with acid-indicator are often not produced, particularly when the acid concentration is very low. A very convenient method has been found for detecting these acids on the filter paper by spraying with a solution containing cupric ions followed by benzidine; also their efficient separation from the mixture has been achieved.

In our experiments α-, β- and γ-pyridine carboxylic acids were used individually or as a mixture. The paper (Whatman No. 1; 15 cm. × 24 cm.) after development (ascending technique) for 4–6 hr. at 30 ± 0·5° C. with the solvent (as described in Table 1) was completely dried by a current of hot air. It was next sprayed with a 0·2 per cent solution of copper sulphate (anhydrous) in a water–ethanol mixture (5 : 4, by vol.) and dried carefully in an oven kept at about 60° C. The dried paper, on spraying with a 0·1 per cent solution of benzidine in 50 per cent ethanol, revealed well-defined bluish spots against a light-brown background. 10–15 μgm. of each component acid could be separated and detected on the chromatogram.

Copper salts, for example, sulphate, chloride, acetate, etc., have been employed as detection reagents; but copper sulphate has given the most satisfactory result. Butanol with a mobile phase acidified with acetic acid gives the best separation and also raises the respective R_F values when compared to butanol alkalized with ammonia[1]. The other solvent systems studied produced high R_F values but contributed little towards resolution. Better results are given when the chamber is maintained fully saturated with respect to the solvent system.

Table 1. R_F Values of Pyridine Carboxylic Acids

Substance	Solvent			
	n-Butanol/acetic acid/water (4 : 1 : 1)	*n*-Butanol saturated with 1·5 *N* ammonia	*n*-Propanol conc. ammonia water (20 : 1 : 4)	Phenol saturated with water
Picolinic acid	0·63	0·15	0·57	0·56
Nicotinic acid	0·89	0·21	0·61	0·88
*iso*Nicotinic acid	0·76	0·22	0·67	0·90

We wish to thank Prof. S. K. Bhattacharyya for his interest and encouragement. Our thanks are also due to Messrs. Sarabhai Chemicals for a gift of *iso*-nicotinic acid.

JIBAN K. CHAKRABARTI
AJOY K. GUHA

Department of Applied Chemistry,
Indian Institute of Technology,
Kharagpur, India.

[1] Huebner, C. F., *Nature*, **167**, 119 (1951).
[2] Kodicek, E., and Reddi, K. K., *Nature*, **168**, 475 (1951).
[3] Leuschner, F., *Naturwiss.*, **40**, 554 (1954).
[4] Hashizume, T., *Nature*, **173**, 645 (1954).

A Simple Method of *Rh* Determination

IT has been shown that about 0·5 per cent of human sera possess a thermostable factor agglutinating *O Rh*+ red cells sensitized with anti-*D* antibodies. Only those sera were taken into account which agglutinated sensitized red cells at least in 1 to 8 dilution in saline. Weaker sera gave unreproducible reactions. The strongest serum observed had a titre of 128. According to our experiments[1], the agglutinating factor in the sera observed is an anti-globulin antibody. It may be absorbed from the sera by sensitized red cells and it may be neutralized in the sera by incomplete antibodies regained at 56° C. from cells. This antiglobulin antibody reacts only with immune antibodies after their denaturation in serological reaction, and therefore it is not neutralized by normal serum or by immune serum containing unchanged antibodies (Fig. 1). The discussion on the nature of this antibody and on its eventual role in pathology will be given elsewhere. Here we wish to stress the practical application of our findings.

(1) A 3 per cent suspension of red cells sensitized with incomplete antibody was tested with human antiglobulin sera. Cells sensitized with all anti-*D* sera gave strongly positive reactions. Tests performed with other incomplete antibodies gave various results depending only upon the strength of the sensitization and not upon the specificity of antibody used. Since the human antiglobulin antibody is not neutralized by serum proteins (other than denatured antibody) the washing of sensitized cells can be omitted.

(2) *Slide agglutination test with mixed serum.* All the incomplete sera may be easily changed into saline agglutinating sera simply by mixing with human antiglobulin sera. After preliminary experiments, the following technique was adopted. Undiluted anti-globulin serum is mixed in equal proportions with undiluted incomplete anti-*Rh* serum. To one drop of mixed serum on a slide is added one drop of 3 per cent saline suspension of red cells with corresponding *Rh* antigen. The slides are allowed to stay in a wet chamber at room temperature for 20–30 min., after which clear-cut results are obtained. The agglutination is a very firm one and does not disappear after vigorous shaking.

In this manner fifteen anti-*D*, three anti-*c*, one anti-*C* and one anti-*e* incomplete sera have been tested. In all cases the mixed serum gave the expected reactions. The controls with cells of non-corresponding *Rh* types were always negative. The titre of a mixed serum was 4–16. The mixed serum can be stored frozen for at least five months. This

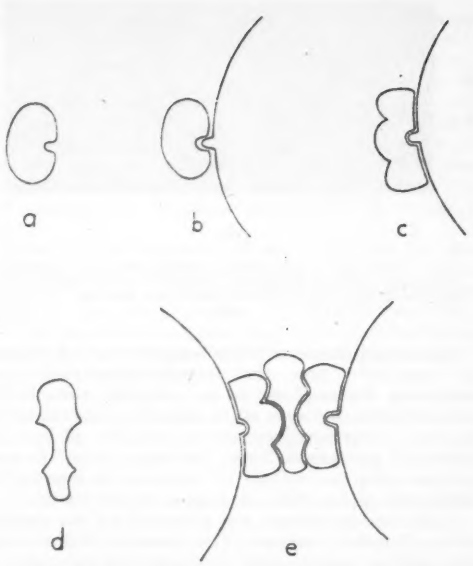


Fig. 1. (*a*) Incomplete antibody; (*b*) serological linkage with red cells; (*c*) denaturation of the antibody molecule in reaction with antigen; (*d*) human antiglobulin antibody; (*e*) agglutination of sensitized red cells

method of *Rh* determination is much simpler than any other used.

(3) *Antiglobulin test with platelets.* The chief difficulty in carrying out the antiglobulin test with platelets (and also leucocytes) is the necessity of saline washing, which causes clumping of these cells. With our human antiglobulin sera, washing can be omitted. Our preliminary experiments are promising.

While our investigation was in progress, we saw the interesting paper in *Nature* of Lewis and Chown[2]. It is now clear that these authors were using a very weak human antiglobulin sera. Therefore they obtained agglutination with mixed sera and could produce this phenomenon only with some anti-*Rh* sera. The negative results with sensitized cells made impossible a correct interpretation of the results.

F. MILGROM
S. DUBISKI
G. WOŹNICZKO

Institute of Microbiology,
Silesian School of Medicine,
Zabrze–Rokitnica, Poland.
March 26.

[1] Milgrom, F., Dubiski, S., and Woźniczko, G., *Vox Sanguinis* (in the press).
[2] Lewis, M., and Chown, B., *Nature*, **173**, 44 (1954).

Carnosine Phosphate as Phosphate Donor in Muscular Contraction

CARNOSINE phosphate was first synthesized by Severin, Georgievskaya and Ivanov[1] by a method very similar in detail to that for creatine phosphate[2]. They showed that this method yields as a rule a diphosphate, both phosphate bonds being high-energy; but that the one which is not on the imidazole ring is more labile. In the presence of muscle extract the latter is transferred to adenosine diphosphate.

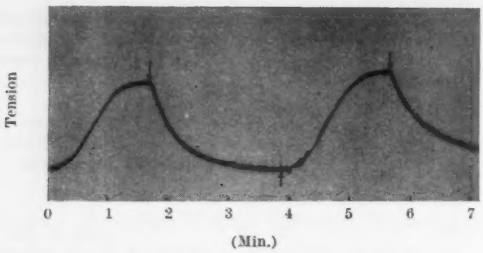


Fig. 1. Isometric tension of extracted muscle fibre in solution containing carnosine phosphates equivalent to 30 millimoles of phosphorus and 4 millimoles of magnesium chloride. ↑ *p*H 7, ↓ *p*H 6

In another direction it has been shown by a number of workers[2–5] now that transphosphorylation to adenosine diphosphate is an essential, though not in itself sufficient[6], part of the relaxation mechanism in muscle. Furthermore, the possibility of an unidentified phosphate donor has been noted[7] in connexion with the failure to observe an increase of adenosine diphosphate during a muscle twitch.

Carnosine phosphate was prepared by the method of the Russian workers. The measured N/P showed that a 50 per cent mixture of di- and mono-phosphates was obtained. A solution containing 30 m*M* phosphorus final concentration was tested by the method of Goodall and A. G. Szent-Györgyi[3] for the relaxing action of creatine phosphate on the glycerol-extracted muscle fibre. The results indicated in Fig. 1 show an effect practically identical with the latter.

The result strongly supports the claim of the Russian workers and raises the question of the presence and role of this substance in muscle. All muscles contain carnosine or anserine (in which the less labile phosphate site is occupied by methyl) in amounts up to 20 m*M*. It was noted in the course of phosphorus-determination that carnosine phosphate is much more acid-labile than creatine phosphate. This may account for its non-detection in muscle so far.

This work was aided by a grant from Muscular Dystrophy Associations of America, Inc., to Dugald E. S. Brown.

M. C. Goodall

Department of Zoology,
University of Michigan,
Ann Arbor, Michigan.
April 12.

[1] Severin, S. E., Georgievskaya, E. F., and Ivanov, V. I., *Biokhimia*, **12**, 35 (1948).
[2] Ennor, A. M., and Stocken, L. A., *Biochem. J.*, **43**, 190 (1948).
[3] Goodall, M. C., and Szent-Györgyi, A. G., *Nature*, **172**, 84 (1953).
[4] Bendall, J. R., *J. Physiol.*, **121**, 232 (1953).
[5] Lorand, L., and Moos, C., *Fed. Proc.*, **15**, 121 (1956).
[6] Kumagai, H., Ebashi, S., and Takeda, F., *Nature*, **176**, 166 (1955).
[7] Fleckenstein, A., Janke, J., Davies, R. E., and Krebs, H. A., *Nature*, **174**, 1081 (1954).

Appearance of Serine upon Alkaline Incubation of Iodinated Polytyrosine

3,5-Diiodotyrosine is known to be a precursor of thyroxine[1,2]. The formation of thyroxine from two diiodotyrosine residues leads obviously to the formation of a three-carbon α-amino-acid. Thus simple dismutation would lead to alanine[2,3], while an oxidative mechanism involving the formation of a labile intermediate of quinonic structure should yield serine[1,3,4]. In the latter case, serine could be formed either by the direct hydrolysis of the labile intermediate[4], or by the hydration of dehydroalanine formed upon molecular dissociation of the labile intermediate[1]. If the unsubstituted 3,5-diiodotyrosine is used in the reaction, dehydroalanine formed may also be hydrolysed to pyruvic acid and ammonia. Johnson and Tewkesbury identified these two products in their reaction mixture[4]. Pitt-Rivers identified alanine among the products of the aerobic incubation of N-acetyl-L-diiodotyrosine chromatographically, but stated that alanine could have been formed afterwards from pyruvic acid[5].

Iodinated polytyrosine was chosen for the reinvestigation of the products of alkaline incubation of diiodotyrosine present in a peptide chain. The high-molecular weight compound seemed suitable for this purpose, as it was expected that the high local concentration of diiodotyrosine residues in the macromolecule might facilitate the reaction between the residues. Furthermore, the protection of both the carboxyl- and the amino-group of 3,5-diiodotyrosine is known to increase the yield of the reaction[2,5].

Poly-L-tyrosine[6] (number average degree of polymerization 30) was iodinated with 0·2 mole of iodine per mole of tyrosine residue, using the iodination technique described previously[6]. The iodinated polymer was hydrolysed with 6 *N* barium hydroxide for 8 hr. at 120°, and the barium was removed as barium sulphate. A paper chromatogram (*n*-butanol/glacial acetic acid/water, 25 : 6 : 25) of the hydrolysate showed on spraying with ninhydrin three spots, identified as tyrosine, monoiodotyrosine and diiodotyrosine.

A 1 per cent solution of the iodinated polymer in a bicarbonate buffer *p*H 10·2 was incubated at 37° for a week. The polymer was precipitated with hydrochloric acid and hydrolysed as above. Paper chromatograms (*n*-butanol/glacial acetic acid/water, 25 : 6 : 25) of the hydrolysate showed on spraying with ninhydrin six spots: the three original spots (tyrosine with R_F 0·36, monoiodotyrosine with R_F 0·58 and diiodotyrosine with R_F 0·66), as well as three new spots, one with R_F 0·17 travelling identically with serine, one with R_F 0·80 travelling identically with thyroxine or 3,5,3′-triiodothyronine, and a faint spot with R_F 0·73. Alanine moves in the chromatographic developer mixture used with R_F 0·23, and in a mixture of alanine with the hydrolysate investigated the spot of alanine could be clearly separated by chromatography from the spot corresponding to serine. Treatment of the chromatogram of the hydrolysate with cerium sulphate–sodium arsenite[7] showed that the compounds travelling with R_F values of 0·73 and 0·80 contain iodine. The hydrolysate was developed also in *n*-propanol/water/concentrated ammonia (100 : 50 : 1) and in water-saturated phenol, and in both cases the chromatograms showed a spot moving identically with serine. In order to confirm the identity of serine on the chromatograms, a larger amount of the hydrolysate was chromatographed and the band corresponding to serine was eluted. A specific serine-requiring mutant *E. coli* K_{12}W–1977, kindly supplied by Dr. J. Lederberg, assayed on a minimal medium described by Tatum and Lederberg[8], responded to the concentrated eluate.

It can be seen from the above that serine, but no alanine, is formed during the alkaline incubation of

iodinated polytyrosine. The spots moving with R_F values 0·73 and 0·80 are probably iodinated thyronines.

Similar experiments have been carried out on poly-L-tyrosine samples of average degrees of polymerization 8 and 50, iodinated with 0·2 and 0·7 mole of iodine per mole of the tyrosine residue respectively. A polymer composed on the average of fifty α-amino-acid residues, of which 14 moles per cent were tyrosine, 20 moles per cent were monoiodotyrosine and 64 moles per cent were diiodotyrosine, contained after incubation 2 moles per cent of serine in the hydrolysate. The amounts of the various α-amino-acids were determined by quantitative ninhydrin colorimetry after chromatography.

We wish to thank Dr. Rosalind Pitt-Rivers for helpful suggestions and for samples of thyroxine and 3,5,3′-triiodothyronine, and Prof. E. Katchalski for his continued interest in this work.

MICHAEL SELA
SARAH SARID

Department of Biophysics,
Weizmann Institute of Science,
Rehovot, Israel.
April 4.

[1] Harington, C. R., *J. Chem. Soc.*, 193 (1944).
[2] Roche, J., and Michel, R., "Advances in Protein Chem.", **6**, 253 (1951); *Physiol. Rev.*, **35**, 583 (1955).
[3] Roche, J., Michel, R., and Nunez, J., *Bull. Soc. Chim. Biol.*, **37**, 229 (1955).
[4] Johnson, T. B., and Tewkesbury, jun., L. B., *Proc. U.S. Nat. Acad. Sci.*, **28**, 73 (1942).
[5] Pitt-Rivers, R., *Biochim. Biophys. Acta*, **2**, 311 (1948); *Biochem. J.*, **43**, 223 (1948).
[6] Katchalski, E., and Sela, M., *J. Amer. Chem. Soc.*, **75**, 5284 (1953).
[7] Bowden, C. H., and Maclagan, N. F., *Biochem. J.*, **56**, vii (1954).
[8] Tatum, E. L., and Lederberg, J., *J. Bacteriol.*, **53**, 673 (1947).

Effect of L-Thyroxine on Carotene and Vitamin A Metabolism in the Cow and the Chick

IT has long been known that vitamin A metabolism is related to the functioning of the thyroid gland[1–3]. The relation between thyroid hormone and fæcal excretion of ingested carotene has been demonstrated by Cama and Goodwin[4] in the rat, and by Chanda *et al.*[5] in the cow and the goat. The effect of the thyroid on the storage of dietary carotene as vitamin A in the liver of rats has also been demonstrated[6]. Chanda and Owen[8] studied the changes in the carotene and vitamin A content of cow and goat milk as effected by parenteral administration of DL-thyroxine. More recently, Chanda, Clapham and Owen[7] have reported their findings on the effect of anterior pituitary thyrotrophic hormone on the simultaneous changes in the partition of carotenoids and vitamin A in the blood serum and the milk of cows.

Since the development of new methods for the synthesis of crystalline L-thyroxine by the Glaxo Laboratories, Ltd., the galactopoietic action of the hormone has been studied by British workers[8]. The present communication reports briefly the effects of synthetic L-thyroxine on carotene and vitamin A metabolism in the cow and the chick.

Two experiments were carried out. In the first, six cows of the Hariana breed were divided into three groups of two each, and the experiment was divided into four periods, the first and last of which served as control periods. Each of a pair of cows received daily by mouth 6 gm. L-thyroxine triturate during period (2), and 8 gm. during period (3). A second pair each received parenterally 7·5 mgm. and 10 mgm. DL-thyroxine per day during periods (2) and (3) respectively. The remaining pair served as controls; but one of them received 6 gm. and 8 gm. lactose per day during periods (2) and (3) respectively, while the second cow received 10 ml. normal saline per day during periods (2) and (3). The hormone was also administered parenterally in 10 ml. solution prepared according to the method of Chanda and Owen[8].

In a second experiment, groups of one-week old chickens of Rhode Island Red and White Leghorn breeds were for eight weeks given a feed mixture containing the hormone; one group received 1 gm. L-thyroxine triturate per 10 lb. feed, and a second group received 20 mgm. DL-thyroxine (mixed in 980 mgm. lactose) per 10 lb. mixture. The diet of the third group of birds, which acted as controls, contained an equivalent amount of lactose only. The triturate contained 1 per cent L-thyroxine. There were ten birds of each breed in each group. Two birds of each breed from each group were killed on the day the feeding of experimental diet started, and, thereafter, at intervals of two weeks. Samples

Table 1. CONCENTRATION OF β-CAROTENE, VITAMIN A ESTER AND VITAMIN A ALCOHOL IN COW BLOOD SERUM AND OF VITAMIN A ESTER IN COW MILK-FAT

Treatment in periods (2) and (3)*	β-Carotene in blood (μgm./100 ml. serum) Period				Vitamin A ester in blood (I.U./100 ml. serum) Period				Vitamin alcohol in blood (I.U./100 ml. serum) Period				Vitamin A ester in milk (I.U./gm. fat) Period			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Saline control	964	942	904	856	23	27	24	19	91	90	90	90	25·2	24·7	24·8	24·2
L-Thyroxine (orally)	973	1,075	1,218	919	26	45	52	24	91	91	97	88	24·7	32·4	39·1	28·5
DL-Thyroxine (parenterally)	991	1,191	1,328	969	26	34	41	21	95	98	108	93	25·5	29·2	32·0	26·9

* There were two cows in each treatment: five determinations were made for each cow during each period. Mean values of the two cows in each period are recorded here.

Table 2. PARTITION OF VITAMIN A IN BLOOD SERUM AND LIVER OF CONTROL AND HORMONE-TREATED RHODE ISLAND RED CHICKS

Treatment	Serum vitamin A alcohol (I.U./100 ml.)			Serum vitamin A ester (I.U./100 ml.)			Liver vitamin A ester (I.U./gm. tissue)			Liver vitamin A alcohol (I.U./gm. tissue)		
	Initial*	Final†	Change	Initial	Final	Change	Initial	Final	Change	Initial	Final	Change
Lactose control	69	115	+46	8	46	+38	42	225	+183	17	51	+34
L-Thyroxine	66	137	+71	10	58	+48	45	290	+245	19	79	+60
DL-Thyroxine	68	140	+72	11	62	+51	46	302	+256	22	86	+64

* One-week old chicks at the commencement of hormone treatment.
† After 8 weeks of hormone treatment.

of blood, liver and intestine from individual birds were analysed separately for carotene and vitamin A.

The partition of carotenoids and vitamin A in milk, blood and other tissues was carried out by the method already described by me and my associates[6,10,11] at the Hannah Dairy Research Institute.

Cows. L-Thyroxine administered orally caused an increase in the β-carotene and vitamin A ester content of the milk fat of Hariana cows in much the same way as was observed previously in the milk fat of Ayrshire cows treated with DL-thyroxine parenterally[6]. It was further observed in the present experiment that the β-carotene and vitamin A ester content of blood serum increased during thyroxine treatment, and that the increases in milk and blood vitamin A ester were greater when the hormone was given orally than when it was given parenterally (Table 1). As no preformed vitamin A was present in the diet of the cows, the latter effect was due probably to greater conversion of dietary carotene into vitamin A in the intestines (see experiment with chicken). The increases in the β-carotene content in the blood (Table 1) and milk due to hormone treatment were greater in cows treated parenterally than in cows which received the hormone orally.

The vitamin A alcohol content of both the milk and blood serum lipids remained fairly constant, except during noticeable hyperthyroidism during period (3), when increases occurred only in the cows treated with hormone. It was further observed that the effects of thyroxine on carotene and vitamin A metabolism were independent of the responses in the milk yield.

Chicken. Vitamin A ester contents of blood and liver determined at two-week intervals showed a gradual increase with age in all the groups, but the rate of increase was accelerated by the hormone treatment. The vitamin A alcohol levels in the blood were also built up at a greater rate in the birds treated with hormone than in the control birds. This is evident from Table 2, in which only the initial and final value for Rhode Island Red birds are recorded for the sake of brevity.

In order to find out whether the greater concentrations of vitamin A in the birds treated with hormone were due to greater conversion of carotene to vitamin A in the intestine, an experiment was carried out in which twelve Rhode Island Red birds were divided into two groups of six each. Both groups received a carotene-free and vitamin A-free ration, but one group received 1 gm. L-thyroxine per 10 lb. feed mixture. The birds were initially four weeks old; the dietary and hormonal treatment were given for four weeks. After the treatment period, two birds of each group received 1 gm. arachis oil only, while the remaining four birds received 10 mgm. β-carotene in 1 gm. arachis oil. The birds were killed 4 hr. after the meal of β-carotene, and the different segments of the intestines were analysed by the methods of Thompson, Ganguly and Kon[12].

The results recorded in Fig. 1 show that the birds treated with thyroxine converted orally administered β-carotene into vitamin A at a higher rate in their small intestines than the controls which were denied the hormone treatment. It seems reasonable to suppose that the greater concentrations of vitamin A in the blood and liver of the treated birds were due mainly to greater conversion of carotene into vitamin A in the intestines (Fig. 1).

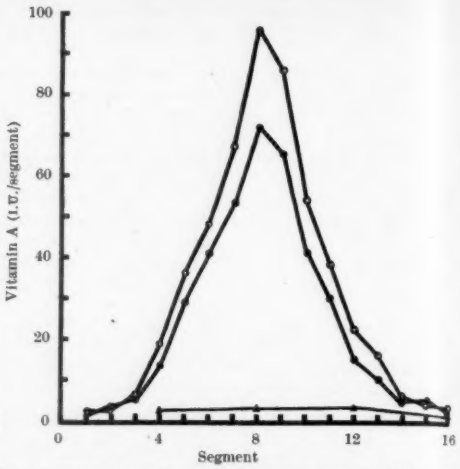


Fig. 1. Vitamin A in the wall of sixteen segments of the small intestine of vitamin A-deficient Rhode Island Red chicks 4 hr. after a meal of 10 mgm. β-carotene in 1 gm. arachis oil (80–90 per cent of the vitamin A was present as vitamin A ester). ○——○, L-thyroxine-treated chick dosed with β-carotene in arachis oil; ●——●, control chick dosed with β-carotene in arachis oil; ▲——▲, L-thyroxine-treated chick dosed with arachis oil (four segments only). Each point in the curves indicates the mean value for four chicks, except that two chicks were dosed with arachis oil only (see text)

The physiological and biochemical significance of these findings will be discussed when full accounts of these and other experiments, which are in progress, are published.

I wish to thank Dr. L. C. Sikka for his interest in this work and Mr. P. B. Sen for permission to use the Unicam *SP*500 spectrophotometer in the Laboratory of the Department of Physiology, University of Calcutta. Thanks are also due to Dr. T. F. Macrae, of Glaxo Laboratories, Ltd., for a generous gift of synthetic sodium L-thyroxine triturate.

R. Chanda
Central Livestock Research-cum-Breeding Station,
Haringhata, P.O. Mohanpur,
District Madia, West Bengal.

[1] Lindquist, T., "Studien über das Vitamin A beim Menschen" (Appelberg, Upsala, 1938).
[2] Drill, V. A., *Physiol. Rev.*, **23**, 355 (1943).
[3] Godwin, T. W., "The Comparative Biochemistry of the Carotenoids" (Chapman and Hall, London, 1952).
[4] Cama, H. R., and Goodwin, T. W., *Biochem. J.*, **45**, 236 (1949).
[5] Chanda, R., Clapham, H. M., McNaught, M. L., and Owen, E. C., *Biochem. J.*, **50**, 95 (1952).
[6] Chanda, R., and Owen, E. C., *Biochem. J.*, **51**, 404 (1952).
[7] Chanda, R., Clapham, H. M., and Owen, E. C., *Biochem. J.*, **60**, 391 (1955).
[8] Leech, F. B., and Bailey, G. L., *J. Agric. Sci.*, **43**, 2 (1953).
[9] Chanda, R., and Owen, E. C., *Biochem. J.*, **50**, 100 (1951).
[10] Chanda, R., *J. Agric. Sci.*, **43**, 54 (1953).
[11] Chanda, R., Owen, E. C., and Cramond, B., *Brit. J. Nutr.*, **5**, 228 (1951).
[12] Thompson, S. Y., Ganguly, J., and Kon, S. K., *Brit. J. Nutr.*, **3**, 50 (1949).

Fluorescence of Adrenaline and Noradrenaline with Ethylene Diamine

Weil-Malherbe and Bone[1] claimed to have estimated the relative proportions of adrenaline and noradrenaline in mixtures of the two by the different colours of fluorescence given by these substances when coupled with ethylene diamine and extracted into *iso*-butanol. We attempted to repeat this work but obtained varying results. We therefore investigated further the mechanism of the reaction.

Oxidation is an essential preliminary: for this, oxygen or other oxidizing agents such as manganese dioxide or potassium ferricyanide may be used; it is not sufficient to rely on the air dissolved in the solutions. Too high a *p*H at this stage reduces the yield of the fluorescent products.

The reaction proceeds in several steps; the relative rates of formation and decomposition of the intermediate products depend on temperature, *p*H and concentration of ethylene diamine.

The first step is the development of a substance having an absorption maximum at 350 mμ. This substance is not fluorescent or appreciably extracted by butanol. Optimum conditions for its formation are *p*H 9·5, ethylene diamine concentration 0·4 per cent (v/v); the yield is maximal in 15 min. (see Fig. 1).

At higher concentrations of ethylene diamine, this substance forms and decomposes rapidly: it gives rise to another substance with an absorption maximum at 405 mμ; an *iso*bestic point is shown at 370 mμ (see Fig. 2). This material is fluorescent and is extracted by butanol, in which solvent it has an absorption maximum at 415 mμ. The optimum conditions for its formation are *p*H 11 and an ethylene diamine concentration of 5 per cent (v/v); at *p*H 10 or 12 the maximum absorption at 405 mμ is only about 60 per cent of that obtained at *p*H 11. The optimum temperature is between 50° and 70° C., the yield being maximal in 1·5–2 hr. Higher concentrations of ethylene diamine make little difference other than to shift the absorption maximum slightly towards a longer wave-length.

This substance with absorption maximum at 405 mμ is comparatively stable. At higher temperatures, higher *p*H, or on prolonged incubation it decomposes to give rise to a product having an absorption maximum at 330 mμ, which is also fluorescent.

Finally, we examined the butanol extracts of the noradrenaline and adrenaline derivatives of ethylene diamine prepared under the conditions which gave the maximum amounts of the substance having an absorption maximum at 405 mμ. These extracts were chromatographed both on filter paper (Whatman No. 1) and on cellulose powder columns; they were developed with water-saturated butanol. A similar result was obtained with either method.

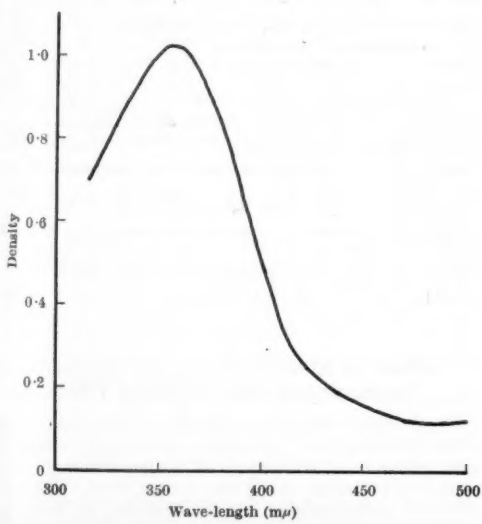


Fig. 1. Absorption spectrum of product having extinction maximum at 350 mμ.
Ethylene diamine 0·4 per cent (v/v) adjusted to *p*H 10·1 with sulphuric acid. Noradrenaline 10·0 μmoles per 100 ml. Solution saturated with oxygen and incubated at 50° for 16 min.

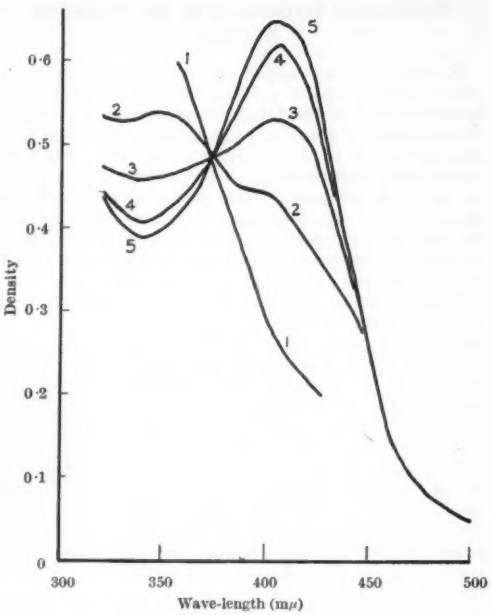


Fig. 2. Time-course of the reaction between ethylene diamine and noradrenaline.
Temperature 51°. Ethylene diamine 3·3 per cent (v/v) adjusted to *p*H 11 with sulphuric acid. Oxygen bubbled through the solution continuously, evaporation being prevented by a reflux condenser. Noradrenaline 7·0 μmoles per 100 ml. added at zero time.
Curve (1), 10 min.; (2) 21 min.; (3) 35 min.; (4) 58 min.; (5) 130 min.

The noradrenaline derivative separated into two fractions: the greater part, R_F 0·64, fluoresced bluish-green; the lesser part, R_F 0·24, fluoresced yellow. The adrenaline derivative also separated into two fractions: the greater part, R_F 0·37, fluoresced yellow; the lesser part, R_F 0·69, fluoresced green.

We have also obtained evidence from the emission spectrum of the noradrenaline derivative that the proportions of the two fluorescent products depend on the concentrations of noradrenaline and of ethylene diamine.

We conclude that there must be at least two fluorescent derivatives of noradrenaline and adrenaline, and that in order to obtain reproducible results the conditions must be very carefully controlled.

We are indebted to Mr. J. R. P. O'Brien for his direction, and Mr. G. Newman for his assistance.

GEORGE P. BURN
E. O. FIELD

Department of Biochemistry
(Radcliffe Infirmary),
University of Oxford.
April 23.

[1] Weil-Malherbe, H., and Bone, A. D., *Biochem. J.*, 57, 311 (1952); *Lancet*, i, 974 (1953).

Spore-like Structures in the Tubercle Bacillus

Electron microscopy of ultra-thin sections of avian tubercle bacilli growing on various solid media has revealed details of their internal anatomy. In the initial phase of growth, the structure was similar to that described in the ordinary bacteria[1-3]. Each rod contained a central dense core in the form of a thread or group of granules within an electron-transparent area (Fig. 1). This structure is believed to represent the nuclear apparatus. There appeared to be some correlation between the phase of growth of the bacillus and the configuration of the dense core. When division by transverse fission took place, each daughter cell contained its own nuclear apparatus. The cytoplasm of the avian tubercle bacilli showed certain differences from the bacteria, and contained large, fairly dense granules (300 A.); but the general pattern of reproduction appeared to be the same.

In later stages of development, under certain conditions, the avian bacilli had a very different internal structure. When grown as slide cultures on thin chicken plasma–embryo extract clots, they did not all divide directly by transverse fission; some of them elongated into filaments, and round bodies were observed forming in the cytoplasm. At first these bodies were difficult to distinguish from the remainder of the cytoplasm; but later they were clearly seen. The nuclear apparatus was still present during the early stages of the formation of the round bodies and was quite distinct from them. The number of round bodies increased until they filled the whole filament except for a peripheral region which contained cytoplasmic granules and dense, metaphosphate bodies (Fig. 2). The round bodies were of uniform internal density and each was enclosed in a dense outer membrane. During the final stages of development some of the filaments contained large vacuoles which appeared to be associated with the formation of the round bodies. Cross-walls were present dividing the filaments into sections each of which contained two or more dense bodies.

In previous studies of unsectioned avian tubercle, rows of vacuoles[4] or intracellular units[5] of varying density and with sharply defined membranes were observed. The interpretation of these units presented some difficulties. They varied in density and were certainly not sap vacuoles as suggested by Knaysi, Hillier and Fabricant[6]; nor could they be identified with the protoplasts[7] of other bacteria, as they were embedded in the cytoplasm. The most likely assumption seemed to be that they were spores, and this was suggested[6]. Our observations on thin sections support this interpretation. From a recent paper by Chapman[8] on spore formation in *Bacillus cereus* and *Bacillus megaterium*, it is seen that the spores in these organisms are strikingly similar to the round bodies in the avian tubercle bacilli.

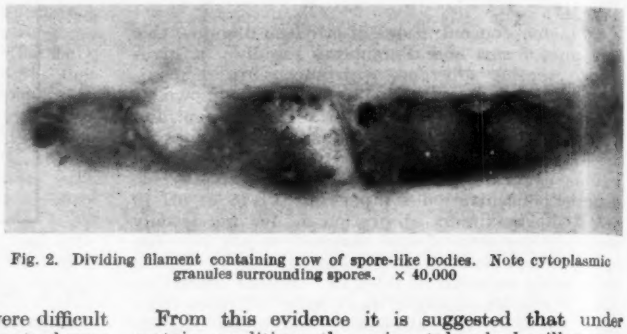


Fig. 2. Dividing filament containing row of spore-like bodies. Note cytoplasmic granules surrounding spores. × 40,000

From this evidence it is suggested that under certain conditions the avian tubercle bacillus produces spores. In future experiments the spore-like bodies will be isolated and their subsequent behaviour studied to test the accuracy of this hypothesis.

We are indebted to Dr. V. E. Cosslett and Mr. R. W. Horne, of the Cavendish Laboratory, Cambridge, for providing electron microscope facilities.

E. M. Brieger
Audrey M. Glauert*

Papworth Hospital and Strangeways Research Laboratory, Cambridge.

* Sir Halley Stewart Research Fellow.

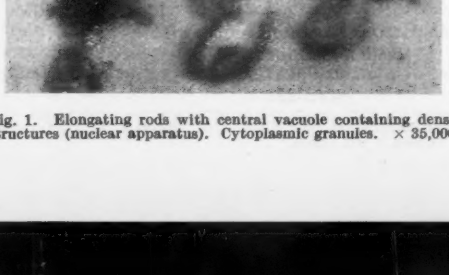


Fig. 1. Elongating rods with central vacuole containing dense structures (nuclear apparatus). Cytoplasmic granules. × 35,000

[1] Chapman, G. B., and Hillier, J., *J. Bact.*, **66**, 362 (1953).
[2] Birch-Andersen, A., Maaløe, O., and Sjöstrand, F. S., *Biochim. Biophys. Acta*, **12**, 395 (1953).
[3] Kellenberger, E., and Ryter, A., *Schweiz. Z. Path. Bakt.*, **18**, 1122 (1955).
[4] Brieger, E. M., Crowe, G. R., and Cosslett, V. E., *Nature*, **160**, 864 (1947).
[5] Brieger, E. M., Cosslett, V. E., and Glauert, A. M., *J. Gen. Microbiol.*, **10**, 294 (1954).
[6] Knaysi, G., Hillier, J., and Fabricant, C., *J. Bact.*, **60**, 423 (1950).
[7] Weibull, C., *J. Bact.*, **66**, 688 (1953).
[8] Chapman, G. B., *J. Bact.*, **71**, 348 (1956).

Effect of Electrolytes on the Rate of 'Supercontraction' of Wool Fibres

Wool fibres have been shown to shorten (supercontract) when held in steam and rapidly stretched and relaxed, or when treated with hot solutions of reagents breaking disulphide bonds, with cold solutions of metal ammonium hydroxides, or with hot solutions of formamide, certain phenols, or lithium bromide (see Alexander and Hudson[1]).

The effects of phenols, formamide, metal co-ordination complexes, and lithium bromide have been

attributed to the rupture of hydrogen bonds, and the effects of most other treatments to splitting of disulphide cross-links. Alexander[2] suggests that lithium ions, being small and having high polarizing power, would be able to combine with polarizable groups of the proteins when the ions are sufficiently concentrated to be incompletely hydrated. He adduces, in support of this theory, the very great increase in rate of supercontraction with increasing concentration of lithium bromide at concentrations corresponding to about ten molecules of water per lithium ion.

In order to gain greater insight into the mechanism of supercontraction in lithium bromide solutions, wool fibres have been immersed in concentrated solutions of various salts buffered at *p*H 6·5–7·0 with 0·1 *M* tartrate or phosphate, maintained for 1 hr. at 100° C., and changes in length measured. Corriedale 56's wool, cleaned by rinsing repeatedly with cold light petroleum, cold ethanol and water, was used for these experiments. The cations investigated were potassium, sodium, lithium, calcium, strontium and beryllium, and the anions, acetate, chloride, bromide, iodide, thiocyanate, trichloracetate and benzoate; supercontraction was obtained with salts containing each of these ions, and several salts, notably lithium and calcium iodide, were more effective than lithium bromide.

The observation by Miss June Griffith (unpublished) that the presence of free halogen in the halide solutions inhibits supercontraction has been confirmed. Removal of free halogen with thiosulphate resulted in supercontraction of the wool at lower salt concentrations and gave greater uniformity of the results.

Curves relating the amount of supercontraction of wool to the concentration of reagent have been constructed for each salt. On arranging the salts of a particular cation in order, according to the concentration required to bring about a certain degree of supercontraction, for example, 25 per cent, the anions have been found to occur in the order of the lyotropic series. With lithium salts the approximate concentrations required for supercontraction of this order were: acetate, $> 12\,M$; chloride, $8{\cdot}5\,M$; bromide, $4{\cdot}5\,M$; iodide, $3\,M$. With a series of iodides the concentrations required were: potassium, $6\,M$; sodium, $5\,M$; lithium, $3\,M$; strontium, $3{\cdot}5\,M$; calcium, $2\,M$.

The results resemble those obtained by Lennox[3] and Jordan Lloyd and Garrod[4] when investigating the effects of a similar series of salts on the thermal shrinkage of collagen. As collagen contains no disulphide linkages and the salts investigated have no known reaction with cystine or its peptides, it is reasonable to conclude that the primary reaction with wool does not involve disulphide bonds but is similar to the denaturation of soluble proteins. The demonstration by Beauregard, Brown and Harris[5] that cross-linking with ethylene dibromide did not prevent supercontraction of wool with lithium bromide also emphasizes this fact.

The above results invalidate Alexander's[2] explanation of the action of lithium bromide, since $3\,M$ lithium iodide, which is as effective as $5\,M$ lithium bromide, contains about seventeen water molecules per cation; also it is unlikely that sodium and potassium ions form complexes with proteins in the manner suggested for lithium ions. An alternative explanation is possible if the tendency for ions to be adsorbed on the wool proteins varies in much the same way as their tendency to become adsorbed on non-protein colloids and on carbon, the small highly hydrated ions being adsorbed least readily and the large, less hydrated ions being adsorbed most readily. The effective molecular charge of a negatively-charged protein would then tend to increase in the presence of high concentrations of a salt of a readily adsorbed anion and a cation which is not readily adsorbed. Any marked increase in molecular charge of the wool proteins would impose stresses on intramolecular and intermolecular bonds, resulting in disaggregation and disordering of the wool proteins. In this regard the effect would be similar to that of anionic wetting agents on the intercellular proteins of the wool cortex[6].

W. G. Crewther
L. M. Dowling

Biochemistry Unit,
Wool Textile Research Laboratory,
Commonwealth Scientific and
Industrial Research Organization,
343 Royal Parade,
Parkville, N.2, Australia. March 21.

[1] Alexander, P., and Hudson, R. F., "Wool: Its Chemistry and Physics", 75 (Chapman and Hall, London, 1954).
[2] Alexander, P., *Ann. N.Y. Acad. Sci.*, **53**, 653 (1951).
[3] Lennox, F. G., *Biochim. Biophys. Acta*, **3**, 170 (1949).
[4] Jordan Lloyd, D., and Garrod, M. E., *Trans. Farad. Soc.*, **44**, 441 (1948).
[5] Beauregard, L. J., Brown, H. E., and Harris, N., *Text. Res. J.*, **23**, 642 (1953).
[6] Crewther, W. G., Proc. Int. Wool Text. Res. Conf. Aust., 1955, *C*, 227 (1956).

Cytotaxonomy and the Migration of Primitive Peoples

A feature of the island-to-island migrations of Polynesian and other Pacific races is that the natives carried with them certain of their cultivated food plants and established them in their new settlements. Some of the original plantations have survived until the present day, and it may be possible to trace the paths of early human migration through a study of the distribution of the food plants.

Certain species of taro (*Colocasia antiquorum* Schott and the closely related (if not synonymous) *C. esculenta* Schott) seem particularly suitable for cytological study since they do not flower readily in many localities and rarely, if ever, appear to set viable seed. The method of propagation is to cut off the base of the rootstock (which is eaten) and replant the upper portion which includes both roots and shoot. Less frequently, in certain varieties, clonal segregates are obtained. Because of these exclusively asexual reproductive modes, the chromosome number is likely to remain constant over long periods.

While the majority of varieties studied to date (some sixty in number) have a diploid chromosome number of 28, the triploid figure of 42 has occasionally shown up. Of particular interest is the fact that a 42-chromosome variety has been found in New Zealand, in a locality (Cavalli Islands) and in circumstances which strongly suggest that it has survived there since the earliest known Maori settlement. A preliminary examination of some twenty varieties from the Polynesian region of the Pacific has failed to bring to light any triploid types. On the other hand, as a result of recent investigations in New Caledonia, two varieties were found with 42 chromosomes.

While it is too early to draw any conclusions from these findings, it is interesting to note that 42

chromosomes have been recorded independently by Japanese and Indian cytologists for the variety *gigantea*. With no evidence so far to the contrary, it is not inconceivable that the New Zealand variety has derived from a south-east Asian source via the Malayan Peninsula and the chain of islands to the south and east.

It must be stressed, however, that the bearing of such evidence, alone, on the origins of the Maori cannot extend beyond the nearest neighbouring island in a chain of this sort, since it is entirely within the realms of possibility that the Maori acquired the taro while *en route* from a totally different area of the Pacific. Nevertheless, by an extension of the method (since presumably the forerunners of the Maori carried comparable food plants in prior migrations) it may be possible to trace the origin of this and other racial groups progressively farther and farther back into prehistorical times. Further studies along these lines are being planned, utilizing in addition other species of taro (*Xanthosoma*, *Alocasia*, *Cyrtosperma*) as well as *Ipomoea* spp. (sweet potato and kumara) and the yam (*Dioscorea*).

J. A. Rattenbury

Botany Department,
Auckland University College,
New Zealand. April 10.

Histamine of the Reptilian Stomach and Lung

Wright *et al.*[1] have demonstrated that the stomach of *Testudo graeca*, *Tachysaurus rugosus* and *Tiliqua nigro-lutea* secretes hydrochloric acid and some pepsin in response to large doses of histamine but is only slightly responsive to vagal stimulation. The histamine concentration of the stomach of *Tiliqua nigro-lutea* (closely related to *Tachysaurus*) was therefore investigated in November 1955. The full thickness of stomach of one lizard was cut up, after scraping off contents, and boiled in Tyrode. The concentration of histamine on titration was 75 μgm./gm., which is a high level.

Histamine was demonstrated in the Tyrode-boiled extract by assay on the isolated jejunum of the guinea pig suspended in Tyrode, before and after treatment with anti-histamine, when the response of equivalent doses of histamine always equalled that of the titrated stomach extract. Assay on the systemic blood pressure of the atropinized cat confirmed the concentration exactly. Further, paper chromatography with two solvents (propyl alcohol–acetic acid–water and butyl alcohol–acetic acid–water) yielded equal runs with pure histamine hydrochloride and stomach extract, one run of which is illustrated in Fig. 1.

Extract for the chromatography was obtained by the modified method of Code[2] for histamine extraction of the whole stomach of another lizard which contained a comparable amount of histamine. Barger and Dale[3] found 19 μgm. histamine/gm. in the intestinal mucosa of the ox, Kellaway and Trethewie[4] a maximum of 48 μgm. histamine/gm. in the whole perfused gut of the guinea pig, and unsqueezed adult guinea pig lung (Australian) contains usually 20–40 μgm. histamine/gm. [5].

The lungs of these reptiles showed no evidence of histamine in them, only a 'slow-reacting' substance like that described by Kellaway and Trethewie[4] or 'slow-contracting substance'[6,7].

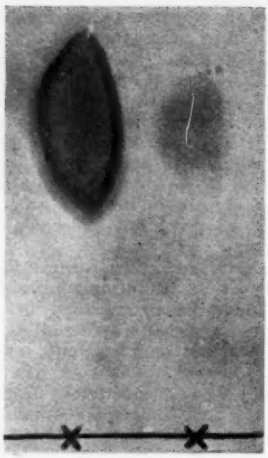


Fig. 1. Chromatogram of lizard stomach extract compared with histamine dihydrochloride, with propyl alcohol – acetic acid – water solvent

The paper chromatography was kindly performed by Mr. Peter Morgan.

R. D. Wright
E. R. Trethewie

Department of Physiology,
University of Melbourne.
March 8.

[1] Wright, R. D., Florey, H. W., and Sanders, A. G. (to be submitted for publication, 1956).
[2] Code, C. F., *J. Physiol.*, **89**, 257 (1937).
[3] Barger, G., and Dale, H. H., *J. Physiol.*, **41**, 499 (1911).
[4] Kellaway, C. H., and Trethewie, E. R., *Quart. J. Exp. Physiol.*, **30**, 121 (1940).
[5] Trethewie, E. R., *J. Immunol.*, **56**, 211 (1947).
[6] Feldberg, W., and Kellaway, C. H., *J. Physiol.*, **94**, 187 (1938).
[7] Trethewie, E. R., *Aust. J. Exp. Biol.*, **16**, 225 (1938).

A Case of DDT Poisoning in Fish

A series of experiments were conducted at Khartoum by a private company concerning the control of *Tanytarsus lewsii*, a green chironomid. During these experiments, a section of the Blue Nile was sprayed by air at Soba Village about ten miles above Khartoum, with an emulsifiable oil containing 30 per cent DDT and 0·5 per cent 'Lissapol'. The equivalent of 440 lb. DDT was sprayed over an area in order to give a calculated DDT content of 0·09 p.p.m. It was hoped that by the time treated water reached Khartoum, the DDT concentration would have been diluted four times. The concentration of DDT at the time of arrival at Khartoum was 0·017 p.p.m. and was then maintained at a level of 0·003 p.p.m. for six hours.

The same day, it was reported that "hundreds of fish were found dying" at Burri Village about two miles above Khartoum, at about the time the treated water was expected to arrive there. Two of these fish were brought to these laboratories on the following day. Prior to chemical examination, they were submitted to Dr. Rzoska, of the University College, Khartoum, for post-mortem examination, who reported the following findings:

"The dead fish was a specimen of *Labea* sp., a plant feeder. The gills were almost white.

"The dying fish was a specimen of *Synodontis schall*, a carnivorous bottom feeder. It was severely bloated.

"The sudden appearance of a large number of dead fish of different species at the same time points to poisoning as the cause of death, and not to disease."

The two fish were then examined for the presence of DDT in different organs using the method of Schechter *et al.*[1] after a preliminary separation of the DDT from the fatty matter using the method of Schechter *et al.*[2]. The following results were obtained:

Fish	Organ	Weight taken (gm.)	Weight DDT found (μgm.)	DDT in organ (p.p.m.)
Labea sp.	gills	4·32	4	0·9
	viscera	14·26	36	2·5
	flesh	12·33	nil	nil
Synodontis schall	gills	7·04	19	2·7
	viscera	26·29	2,080	79
	fatty deposit	1·37	87	64

These results lend some support to previous work showing that fish are highly sensitive to DDT, and that non-fatty animals are more sensitive than fatty animals (summary in Hinton[3]). The *Labea* was found dead some eight hours after spraying, whereas the *Synodontis* survived thirty-six hours.

However, the possible effect of the surface-active agent should not be overlooked.

E. H. W. J. Burden
(Government Analyst)

Wellcome Chemical Laboratories,
Ministry of Health,
Khartoum.
April 30.

[1] Schechter, M. S., Soloway, S. B., Hayes, R. A., and Haller, H. L., *Indust. Eng. Chem.*, Anal. Ed., **17**, 704 (1945).
[2] Schechter, M. S., Pogorelskin, M. A., and Haller, H. L., *Ind. Eng. Chem.*, Anal. Ed., **19**, 51 (1947).
[3] Hinton, H. E., *Sci. Prog.*, **43**, No. 172, 643 (1955).

Occurrence of Paracolon Bacteria in a Tropical Marine Environment and their Classification

Late or non-lactose fermenting bacteria are of wide interest in the clinical field because of their association with gastroenteritis and urinary infections. Because they form colourless or white colonies on coliform differential media, they are usually overlooked during the isolation of coliforms. Clemesha[1] was probably the first to recognize the importance of these organisms in India, when he stated that "a class of organisms which ferment glucose but not lactose is not only exceedingly common in water . . . but the significance of these bacteria is of great importance to the water analyst". Topley and Wilson[2] also felt that "there seems no doubt that they are a more common constituent of normal intestinal flora under tropical than under temperate conditions". In India these 'Paracolon' bacteria are misclassified in the genus *Escherichia* or *Aerobacter* despite the fact that lactose fermentation is a fundamental characteristic of the latter organisms. Topley and Wilson[2] defined 'Paracolons' as those which do not ferment lactose or ferment it slowly and have the 'IMViC' reaction + + − −. Stuart *et al.*[3] suggest the term 'Paracolon' for papillæ-forming anaerogenic and slow lactose-fermenting *Escherichia*-like organisms associated with gastro-enteritis. Breed *et al.*[4] have accorded generic rank to *Paracolobactrum*, including them as an appendix to the tribe Escherichiae.

While working on the coliform types in green mussels[5], we isolated some 'paracolons' also. Two are similar to *Paracolobactrum aerogenoides*, whereas eight others belonged to *Paracolobactrum coliformae*. Three of these were malonate-positive but differed from those of Schaub[6]. All the cultures fermented glucose and all but one fermented sucrose. Dulcitol was not fermented by any of them whereas a few fermented adonitol. Gelatin liquefaction was not common, neither did many produce hydrogen sulphide. Lactose fermentation was mostly negative and very delayed if positive. All the *Escherichia*-like paracolons produced indole, in agreement with the findings of Kligler[7] and Ferguson and Wheeler[8]. Though *MR*-positive coliforms ferment glycerol, only two of the *Paracolobactrum coliformae* fermented glycerol. A few cultures were weakly urease positive in Christensen's medium but none in Rustigian and Stuart's, thus excluding their relationship to *Proteus*. Our cultures were all peritrichous when very young (5 hr. old) but they showed 'degenerate peritrichous' flagella when 18 hr. old.

Breed (personal communication) would include polar flagellate glucose-fermenting (aerogenic) but non-lactose fermenting organisms in the new genus *Aeromonas* Kluyver and Van Niel. The culture described by us as *Paracolobactrum coliformae* var. *marinum*[9] was found later to be monotrichous and would belong to *Aeromonas* but for the fact it is *MR*-positive. Flagellation is an important diagnostic characteristic in distinguishing the Paracolon-Aerobacter from *Aeromonas*. The failure of some coliform bacteriologists to determine the type of flagellation has led to misclassification of *Aeromonas* as *Paracolobactrum*, as emphasized by Breed. Sen and Dutt[10], describing a lactose anaerogenic but glucose aerogenic organism, identified it as *Aerobacter cloaceae*, though it should properly be classified as *Paracolobactrum aerogenoides* (or as *Aeromonas*, if flagellated at the poles). In view of the reported role of paracolons in the etiology of gastric infections, and that of *Aeromonas* in causing diseases of cold-blooded animals, more attention should be paid to their proper classification.

Detailed descriptions of the paracolon cultures will be published elsewhere. We thank Dr. Robert S. Breed for useful discussion and for a preprint of the section on the genus *Aeromonas* prepared for the 7th edition of "Bergey's Manual".

This communication is published by permission of the additional director of Industries and Commerce, Madras.

A. Sreenivasan
R. Venkataraman

Fisheries Technological Station,
Kozhikode 1, S. India.
March 28.

[1] Clemesha, W. W., "The Bacteriology of the Surface Waters of the Tropics" (Thacker and Spink, Calcutta, 1912).
[2] Topley and Wilson, "Principles of Bacteriology and Immunity" (Arnold, London, 3rd edit., 1946).
[3] Stuart, C. A., Baker, M., Zimmermann, A., Brown, C., and Stone, C. M., *J. Bact.*, **40**, 101 (1940).
[4] Breed, R. S., Murray, R. G. E., and Hitchens, A. P., "Bergey's Manual of Determinative Bacteriology" (6th edit., 1948).
[5] Venkataraman, R., and Sreenivasan, A., *Ind. J. Fish.*, **2**, 314 (1955).
[6] Schaub, I. G., and Foley, K. M., "Diagnostic Bacteriology" (C. V. Mosby, 1952).
[7] Kligler, I. J., *J. Bact.*, **4**, 35 (1919).
[8] Ferguson, W. W., and Wheeler, W. E., *J. Bact.*, **51**, 107 (1946).
[9] Venkataraman, R., and Sreenivasan, A., *Curr. Sci.*, **22**, 120 (1953).
[10] Sen, A. N., and Dutt, M. A., *J. Sci. Ind. Res.*, **14**, C, 53 (1955).

Availability to Dinitrofluorobenzene of some Amino Groups in Human Dentin and Ox Hide Collagen

Human dentin (containing 80 per cent by weight of mineral material), ox hide collagen, and samples of dentin which were partially demineralized at 4° C. by soaking in either 1 *N* hydrochloric acid or 1 *N* trichloracetic acid, or 15 per cent versene (*p*H 7·3), were assayed for α- and ε-amino groups using Sanger's dinitrofluorobenzene method[1].

0·2 gm. of tissue was added to 0·5 gm. dinitrofluorobenzene (excess) in 66 per cent ethanol saturated with sodium bicarbonate and shaken for 36 hr. at room temperature. The dinitrophenyl protein thus formed was washed with water and ethanol, and hydrolysed for 16 hr. with 6 *N* hydrochloric acid in a sealed tube at 105° C. After extraction of the hydrolysate with peroxide-free ether, separation of the water-soluble ε-N dinitrophenyl lysine and ε-N dinitrophenyl hydroxylysine was achieved by means of paper-chromatography, using butanol/acetic acid/water (4 : 1 : 5) as solvent. The yellow spots were cut out, eluted into 5 ml. of 1 *N* hydrochloric acid, and their optical densities at 360μ recorded on the Beckman spectrophotometer.

Distinct differences between the availability of amino groups of dentin and those of similarly treated hide collagen were observed :

(1) Only 50–60 per cent of the total lysyl and hydroxylysyl ε-amino groups of hide collagen were available to the dinitrofluorobenzene[2], whereas in human dentin the availability of these amino groups increased linearly from 6 per cent in untreated dentin to more than 90 per cent in the fully demineralized dentin[3].

(ii) A linear increase (from zero to 10 micromoles/gm. protein) in the concentration of N-terminal amino groups during demineralization of dentin was observed. In accordance with other workers[2], no N-terminal amino groups were detected in hide collagen.

(iii) The molar ratio (*R*) of ε-N dinitrophenyl lysine to ε-N dinitrophenyl hydroxylysine, obtained from the dinitrophenylation of hide collagen, was almost theoretical (3·9) [4]; but in dentin collagen this ratio increased in a linear manner from 0·8 to 2·9 (theoretical maximum 3·7) [5], when plotted against the percentage dissolution of mineral material (Fig. 1). This implies that, especially in the early stages of the decalcification process, the availability to dinitrofluorobenzene of hydroxylysyl ε-amino groups exceeds that of the lysyl ε-amino groups, in spite of the much higher concentration of lysine residues. The same results were obtained using each of the above-mentioned demineralizing solutions.

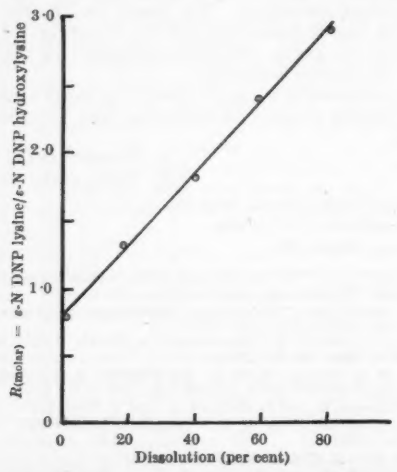


Fig. 1. Relative availability, to dinitrofluorobenzene, of lysyl and hydroxylysyl ε-amino groups of dentin collagen, during mineralization

Other workers[6] have suggested that polar side-chains of amino-acid residues in proteins and peptides may act as binding sites for ions; and since the anionic binding power of the ε-amino groups of hydroxylysine is weaker than that of lysine[7], bound anions are expected to be liberated at a greater rate from hydroxylysyl than from lysyl amino groups, during demineralization.

It is therefore suggested that the ε-amino groups of lysine and hydroxylysine may play a part in the combination of mineral material with the protein matrix of human dentin.

C. C. Solomons
J. T. Irving

Joint Dental Research Unit of the
Council for Scientific and Industrial Research
and the University of the Witwatersrand,
Johannesburg.

[1] Sanger, F., *Biochem. J.*, **39**, 507 (1945).
[2] Bowes, J., and Moss, J., *Biochem. J.*, **55**, 753 (1953).
[3] Solomons, C. C., and Irving, J. T., *S.A. J. Med. Sci.*, **20**, 144 (1955).
[4] Bowes, J., and Kenten, R., *Biochem. J.*, **43**, 358 (1948).
[5] Hess, W., and Lee, C., *J. Dent. Res.*, **33**, 62 (1954).
[6] Klotz, I., Cold Spring Harbor Symp. on Quant. Biol., **14**, 97 (1950).
[7] Klemperer, F., Hastings, B., and Van Slyke, D., *J. Biol. Chem.*, **143**, 433 (1942).

Correlation between Deoxyribonucleic Acid Content and Volume of Individual Nuclei in Different Tissues of the Rat

It has been shown that in the liver[1], and in several other organs[2], of the rat the nuclear classes determined according to the deoxyribonucleic acid content correspond to the nuclear volume classes. An objective analysis was performed in order to see if in the individual nuclei of a given organ a correlation exists between deoxyribonucleic acid content and volume. The results of measurements obtained with E. Pisi[2] were used in this study. The relative deoxyribonucleic acid content of the individual nuclei was obtained by means of the histophotometrical method of L. Lison[3]. The great majority of the nuclei being practically spherical in shape, the nuclear volume was calculated by a simple geometrical formula from the projection surface.

Fig. 1 summarizes the results obtained for the thyroid gland for two rats (105 and 106). The regression lines and the correlation coefficients *r* (0·816 and 0·536) show that, if the degree of correlation can be varying in the same organ from one individual to another, in each case a real linear correlation exists between the deoxyribonucleic acid content of the different nuclei and their volume.

Similar results were obtained for other organs, which like the thyroid gland contain practically only one nuclear class (kidney, endocrine pancreas). In organs with different nuclear classes (liver, exocrine pancreas) conditions seem to be more complicated; they will be the subject of a further study.

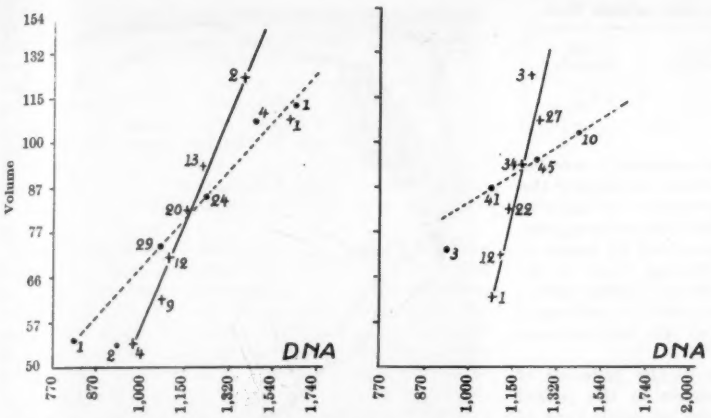


Fig. 1. **Correlation between deoxyribonucleic acid content and nuclear volume in the thyroid gland of the rats 105 and 106: regression lines. Left: Thyroid gland of rat 105; *r*, 0·816; *n*, 61. Right: Thyroid gland of rat 106; *r*, 0·536; *n*, 99. *n*, number of measurements (the number of measurements from which the corresponding mean value was calculated are indicated next to each point); *r* = correlation coefficient**

These investigations were carried out with a grant from the Fonds National de la Recherche Scientifique.

J. FAUTREZ

Laboratory of Human Anatomy and
Belgian Centre of Normal and Pathological Growth,
University of Ghent.

[1] Fautrez, J., Pisi, E., and Cavalli, G., *Nature*, **176**, 311 (1955).
[2] Fautrez, J., and Pisi, E., *C.R. Assoc. Anat.* (in the press).
[3] Lison, L., *Acta Anat.*, **10**, 333 (1950).

Human Fœtal Ventricular Outputs

In a comprehensive study of the fœtal circulation in the lamb, G. S. Dawes, Joan C. Mott and J. G. Widdicombe[1] showed that in the fœtus the left ventricular output exceeded that of the right ventricle. In a study of the ventricles of the post-natal human heart[2], I showed, by observations of the weight of the ventricular mass and of its parts, that right ventricular atrophy is the normal event in the period immediately following birth, and that the weight ratio between right and left ventricles which is characteristic of adult hearts is normally found from the age of six months onwards. Additional unpublished observations on the thickness of the ventricular walls during the period of right ventricular atrophy suggest that in the human fœtus, as in the lamb, the output of the left ventricle exceeds that of the right. As experimental observations of the type performed on the fœtal lamb are unlikely to be made on a human fœtus, these observations may be of some interest.

The measurement of the thickness of the ventricular walls is unsatisfactory both in theory and in practice. One big difficulty is that of making observations at strictly comparable points on the ventricular walls of different hearts. For this and other reasons, I originally determined to rely only on observations of the weight of the myocardium in the survey quoted above. However, when it became obvious that right ventricular atrophy was a normal post-natal event, I began to measure the thickness of the ventricular walls in order to compare changes in such measurements with the changes in weight. In order to obtain reasonably comparable observations, the measurements were made with flat-bladed callipers reading to 1/10 mm. at five points. These were: (*a*) anterior wall of the infundibulum, and (*b*) across the infundibulo-ventricular crest (crista supraventricularis). In both these measurements one blade of the callipers was inserted through the pulmonary orifice of the right ventricle, parallel to the interventricular sulcus in the first, and parallel to the atrioventricular sulcus in the second. (*c*) The posterior wall of the right ventricle—calliper inserted into the tricuspid orifice posteriorly and directed parallel to the interventricular sulcus on the inferior surface of the heart. (*d*) The lateral wall of the left ventricle, between the anterior and posterior groups of papillary muscles. (*e*) The thickest part of the septum.

In each of these measurements the callipers included the trabeculæ and avoided the bases of the papillary muscles. The measurements were made on specimens which had been fixed in 10 per cent formol-saline for 2–3 days.

In practice the septal and left ventricular wall measurements always resembled each other closely. For this reason only the measurements of the free wall of the left ventricle are quoted. Table 1 gives a summary of the findings at four age periods.

Table 1. MEAN THICKNESS OF VENTRICULAR WALLS IN MM.

Age period	No. of specimens	Right ventricle (*a*)	(*b*)	(*c*)	Left ventricle (*d*)
Full term	19	4·2	6·1	4·1	5·2
4–6 months	12	2·5	3·8	2·9	8·0
Second year	8	2·9	4·4	3·0	9·0
Sixth year	4	3·8	6·1	3·9	10·4

The infundibulo-ventricular crest is always by far the thickest part of the right ventricular free wall, and in the fœtus is normally thicker than any part of the left ventricular wall or septum. The atrophy of the right ventricular wall in the early months is profound and is not made up, in thickness, until six years have passed. In contrast the left ventricular free wall and septum grow rapidly in thickness from the beginning.

An estimate of the surface area of either ventricular free wall may be obtained by setting its weight (used as a measure of the volume of the myocardium) against its thickness. In the case of the right ventricle, the weight of its free wall in gm. was divided by its thickness, measured across the infundibulo-ventricular crest, in mm., and for the left ventricle the weight of its free wall divided by its thickness. The resultant figures are arbitrary, and are only used for comparing one heart with another, or one group of hearts with another. These two calculations were made for each specimen, and taking the first two age groups summarized above, give mean estimates of surface area as shown in Table 2.

Table 2. Mean Estimate of Surface Area of Free Wall

Age period	No. of specimens	Right ventricle	Left ventricle
Full term	19	1·0	1·5
4–6 months	12	1·5	1·9

In an irregular cavity such as a cardiac ventricle, surface area will be related to volume in roughly the same way as in a sphere, where volume increases as the cube of the radius, and surface area as its square. Therefore, changes in surface area may be made to indicate changes in volume by raising them to the power of 3/2. Thus the increase in the volume of the right ventricle in the period chosen would be indicated by $(1{\cdot}5/1{\cdot}0)^{3/2} = 1{\cdot}8$, and that of the left ventricle by $(1{\cdot}9/1{\cdot}5)^{3/2} = 1{\cdot}4$.

This suggests greater increase in the capacity of the right than of the left ventricle in the period chosen. At the period 4–6 months an adult type of circulation with equal ventricular outputs is certain. The inference can thus be drawn that the functional capacity of the right ventricle before birth is less than that of the left ventricle. As the fœtal ventricles pump against the same peripheral resistance, a lesser capacity implies a lesser output. This conclusion is in accord with the belief that the general pattern of the circulation shown in the fœtal lamb is repeated in the human fœtus.

E. N. Keen

Department of Anatomy,
University of Cape Town.
May 19.

[1] Dawes, G. S., Mott, Joan C., and Widdicombe, J. G., *J. Physiol.*, **126**, 563 (1954).
[2] Keen, E. N., *J. Anat.*, **89**, 484 (1955).

Delayed Implantation in the Grey Seal, *Halichoerus grypus* (Fab.)

Early in March 1870 a large grey seal cow was killed by Prof. W. Turner. "A large corpus luteum was in the left ovary but the uterus was empty[1]."

On January 3, 1956, we killed a grey seal cow on the Pembrokeshire coast. A moderate-sized corpus luteum, containing a central cavity, was found in the left ovary (Fig. 1). Externally the uterus showed no sign of pregnancy; but, in the left uterus, a blastocyst was found measuring 2·3 mm. by 1·8 mm., lying free in the lumen (Fig. 2). Its cells were virtually free of mitotic figures. The surrounding uterine epithelium and glands showed no evidence of impending implantation.

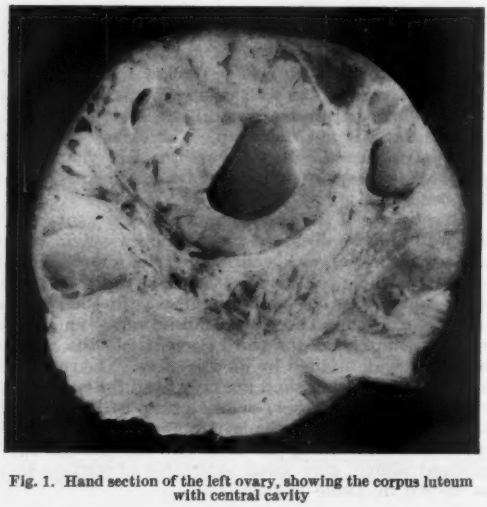


Fig. 1. Hand section of the left ovary, showing the corpus luteum with central cavity

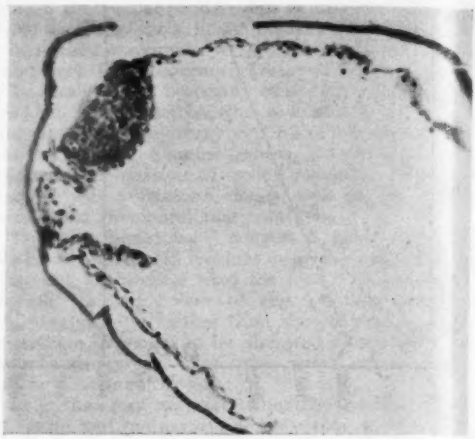


Fig. 2. Vertical section through the blastocyst, showing the zona (broken at one point), the trophoblast, primary endoderm and the inner cell mass

Three bulls all more than 90 in. in total length were shot at the same time. Two of these had the neck and shoulder development characteristic of sexually mature males. There were no sperm, however, in the testes or in the epididymes of any of the three bulls. Fertilization must therefore have taken place some time previously. Mating on the Pembrokeshire coast has been observed during September–October. Consequently delayed implantation seems apparent. Turner's original observation supports this conclusion.

Delayed implantation of the blastocyst has been previously recorded in two other Phocid seals; in the common or harbour seal by Fisher in 1954 [2] and in the elephant seal by Gibbney in 1953 [3].

K. Backhouse

Department of Anatomy,
St. Bartholomew's Hospital Medical School,
H. R. Hewer

Department of Zoology,
Imperial College of Science and Technology,
London, S.W.7.

[1] Turner, W., *J. Anat.*, **4**, 270 (1870).
[2] Fisher, H. D., *Nature*, **173**, 879 (1954).
[3] Gibbney, L., *Nature*, **172**, 590 (1953).

A Culture Technique for *Venturia* spp. and a Turbidimetric Method for the Estimation of Comparative Sporulation

Growth and sporulation of *Venturia inaequalis* and *V. pirina* have been extensively studied in artificial culture. Miller[1] obtained objective measures of sporulation and also concluded that inoculum concentrations greater than 200,000 conidia per ml. had little effect on the final yield of conidia. Montgomery and Moore[2] grew cultures of *V. inaequalis*

on sterilized apple twigs from which the bark had been removed. They obtained rapid growth and sporulation, and could remove most of the conidia easily without disintegration of the substrate, by shaking the cultures with water. In the following methods the advantages of a firm substrate have been coupled with the use of media of known or constant composition for studies of comparative sporulation under various nutritional conditions, and for the provision of routine supplies of conidia for fungicide bioassay.

The cultures are grown on filter paper cylinders standing in nutrient solutions. Whatman 541 filter paper is cut into rectangles of a size to produce, when rolled, cylinders of convenient length and diameter. These cylinders are then slipped into and held centrally by stainless steel rings. The dimensions I have used are 4·5 cm. × 3·5 cm. rectangles, rolled into cylinders 4·5 cm. long, and held by stainless steel rings as follows: outer diameter 5/16 in., thickness 1/64 in. and width 1/32 in. The cylinders are placed in 6 in. × ½ in. B.S.S. standard test-tubes and heat-sterilized before addition of nutrients. Culture media at *p*H 5·6 (for example, 10 per cent malt extract, as suggested by Miller[1]) are sterilized by filtration and 1-ml. aliquots are then dispensed aseptically into each tube.

Inoculum suspensions of conidia or mycelial fragments are prepared in sterile glass-distilled water. Each suspension is strained through fine organdie muslin into a 5 in. × ½ in. test-tube packed with cotton wool, inside a 6 in. × 1 in. boiling-tube. After filtration the muslin is removed and the plug of the outer tube replaced. Filtration is necessary owing to the otherwise inevitable blockage of pipettes, used for dispensing inoculum, by large mycelial fragments. Inoculum concentration is adjusted visually to an approximate turbidity of 0·1 (see below), which is equivalent to 2–300,000 conidia per ml. Each tube is inoculated with a volume of suspension calculated as sufficient just to cover the paper cylinders (0·2 ml. for the dimensions given above) and the tube is shaken and rotated to ensure even distribution. The cultures are incubated horizontally for 14–21 days at 18° C. They may be killed by the addition of two drops of formalin and stored at 5° C. to await estimation of sporulation.

Comparative sporulation is determined by turbidimetric estimation of suspensions produced from each culture, in order to avoid time-consuming counts of spore concentrations and the use of sampling methods. The following procedure has been adopted for the preparation and estimation of suspensions.

Cultures of *V. inaequalis* are shaken vigorously for 1 min. with distilled water (6 ml.) and those of *V. pirina* gently for the same time. Vigorous shaking of the latter is undesirable as they produce comparatively few conidia, and the strongly segmented subaerial mycelium tends to disintegrate. Each spore suspension is strained through one thickness of fine organdie muslin into a 6 in. × 1 in. boiling-tube. The process is repeated with a further 6 ml. of distilled water and gentle shaking. This treatment yields 10–12 ml. of suspension, containing 95–99 per cent of the conidia and a few small fragments of mycelium. Each suspension is centrifuged at 2,000 rev./min. and the supernatant liquid replaced by distilled water; this is repeated until no trace of colour remains in the liquid, in order to avoid interference with the turbidimetric estimation. The final spore deposit is resuspended in either 1 ml. or 10 ml. of distilled water, depending on its magnitude, and the suspension stored at 5° C. in the presence of formalin, to prevent microbial deterioration.

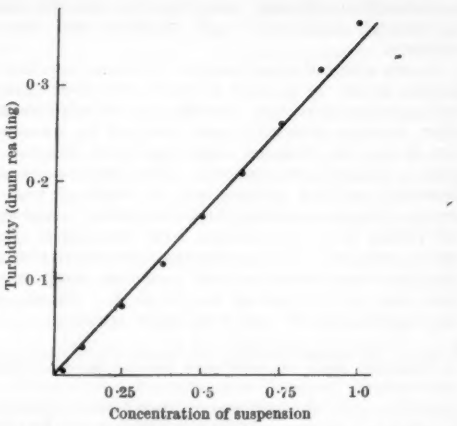


Fig. 1. Linear relationship between dilutions of a concentrated spore suspension and turbidity

The turbidity of each suspension is recorded using a Hilger photoelectric absorptiometer (Spekker) with neutral filters and cells of 1 cm. path-length, microcells being used for the 1-ml. suspensions. An examination of the relationship between dilutions of a concentrated spore suspension and turbidity showed it to be almost linear over the range of drum readings 0·02–0·3; but beyond this range there was an increasing departure from linearity (Fig. 1). Concentrated 10-ml. suspensions are therefore adjusted by dilution to give readings in the 0·02–0·3 range and the necessary corrections applied to give directly comparable estimates of spore production by the cultures. Readings below 0·02 cannot be accurately determined, and 1-ml. suspensions are therefore prepared, the observed drum readings being divided by 10 to obtain results comparable with those of the 10-ml. suspensions. All results are expressed as comparable turbidities.

Results obtained by the use of these methods have been briefly reported by Kirkham[3], and Kirkham and Flood[4], and will be described in detail elsewhere.

D. S. Kirkham

East Malling Research Station,
Nr. Maidstone, Kent.
May 30.

[1] Miller, H. J., *Phytopathology*, **39**, 245 (1949).
[2] Montgomery, H. B. S., and Moore, M. H., *J. Pomol.*, **15**, 253 (1937).
[3] Kirkham, D. S., *Nature*, **173**, 690 (1954).
[4] Kirkham, D. S., and Flood, A. E., *Nature*, [**178**, 422; 1956].

Intake of Streptomycin by Peach Leaves

Previous work[1] has shown that streptomycin sulphate, when applied as an aqueous spray to foliage, prevented the development of blast of stone fruit, caused by *Pseudomonas syringae* van Hall. Mitchell *et al.*[2] found that streptomycin was taken in and translocated upwards to the leaves when applied in a lanolin paste to bean plant stems. As part of a programme designed to gain knowledge of the movement of streptomycin in peach trees (*Prunus persica* (L) Batsch), investigations were carried out

to determine whether streptomycin can be taken in through uninjured leaf surfaces and become systemic.

In one series of experiments, seedlings (var. Golden Queen) grown in pots in a glasshouse were sprayed with aqueous solutions of streptomycin sulphate and, after varying intervals, were washed by immersing the foliage in running water for four minutes, to remove surface streptomycin. The presence of antibacterial activity subsequent to washing was determined by inoculating the plants with *Ps. syringae*. All plants in an experiment were inoculated on the same occasion. The percentage infection obtained when a concentration of 100 μgm./ml. streptomycin base was used is given in Table 1. Plants were dry approximately one hour after spraying.

Table 1. *Ps. syringae* Infection of Peach Leaves when washed at Intervals after Spraying with 100 μgm./ml. Streptomycin

	Time after treatment before plants were washed				Differences for significance at 1 per cent
	Check	15 min.	2 hr.	3 days	
Per cent leaf infection*	100	97	89	65	
Equivalent angle	90	84	75	54	21
Mean lesion diameter (mm.)	10·7	11·6	9·5	7·3	3·2

* Number of lesions out of 20 inoculation points on each of five plants.

Since a time-interval between spraying and washing was required before infection was significantly reduced, it would seem that washing removed surface streptomycin; and that the significant reduction in infection with plants washed three days after spraying was due to streptomycin retained by the leaves. This experiment has been repeated with substantially the same results. Similar results were obtained when leaf tissue extracts from similarly treated plants were assayed by the paper-disk plate method, using *Bacillus subtilus* A.T.C.C. 6633 as test organism. The only extracts which inhibited the test organism were from plants washed three days after spraying.

More conclusive evidence that streptomycin was taken into leaf tissues was obtained by painting solutions containing 1,000 μgm./ml. streptomycin on the under surfaces of leaves for three consecutive days, followed by washing the plants and inoculating upper leaf surfaces. In one such experiment, 11 lesions developed on treated leaves and 71 on untreated leaves.

Preliminary investigations indicate that the time required for streptomycin sulphate to enter leaves in effective or measurable quantities is influenced by the climatic conditions; for example, the period was reduced when treated plants were held at high (> 90 per cent) relative humidity.

When aqueous solutions of streptomycin sulphate containing up to 1,000 μgm./ml. streptomycin base were painted on proximal or distal half leaves, and plants placed in an atmosphere of greater than 90 per cent relative humidity, no antibacterial activity was detected in untreated areas by either bioassay or plant infection techniques. Activity was detected, however, in treated and washed half-leaves of the same plants. No indications of streptomycin translocation were obtained when a colloidal streptomycin formulation with high lipoid solubility properties was painted on half leaves or sprayed on half plants.

When whole peach leaves, except for the petioles, were placed in aqueous solutions of streptomycin, no reduction in streptomycin concentration was detected by bioassay, but when macerated peach leaf tissue was added to streptomycin solutions, the amount of activity detected in the supernatant liquid was considerably reduced[3]. The reduction was not due entirely to dilution or to a change in *p*H.

Charles[4] suggested that xylem elements may be negatively charged and therefore interfere with translocation of positively charged streptomycin molecules.

The work outlined above indicates that streptomycin can enter peach leaves following foliar applications, but no evidence can be found that it becomes truly systemic when applied to leaves. These findings are not inconsistent with the view that streptomycin may become bound in leaf tissues in some way following foliar sprays.

I am grateful to Dr. H. R. Thompson for statistical analyses and to Glaxo Laboratories, England, for supplying the colloidal streptomycin formulation.

M. H. Dye

Plant Diseases Division,
Department of Scientific and
Industrial Research,
Auckland, New Zealand.
April 9.

[1] Dye, D. W., and Dye, M. H., *N.Z. J. Sci. Tech.*, A, **36**, 21 (1954).
[2] Mitchell, J. W., Zaumeyer, W. J., and Anderson, W. P., *Science*, **115**, 114 (1952).
[3] Dye, M. H., *Ann. App. Biol.* (submitted for publication).
[4] Charles, A., *Nature*, **171**, 435 (1953).

Effects of Temperature and Length of Day on the Sex Expression of Monoecious and Dioecious Angiosperms

It has been demonstrated[1] that production of female flowers in *Cucurbita pepo* is delayed when plants are grown in high temperatures and during long photoperiods. Previous investigations indicated a similar effect of temperature and length of day on the sex of *Ambrosia*[2].

In natural summer conditions monoecious plants of *Mercurialis ambigua* L. fil. produce one female flower and a cluster of male flowers in each leaf axil. Ten plants were grown in 24-hr. photoperiods and thirteen in 8-hr. photoperiods throughout their lives at day temperatures of 26°–32° C. and night temperatures of 17°–21° C. All plants produced male flowers from the second foliar node upwards; but long days significantly delayed the production of female flowers. The average degree of femaleness attained at each node in each group of plants, as a percentage of the maximum possible, is given in Table 1. 100 per cent of femaleness at a node is reached when all plants of the group have two female flowers at that node.

In a second experiment, plants were grown in continuous light (8 hr. natural daylight supplemented by 16 hr. light at 500 ft. candles from a mercury lamp). Twelve of these plants were grown at a constant temperature of 24° C. and another twelve at 15·5° C.

Table 1. Average Percentage of Femaleness

Day-length	Node numbers 2	3	4	5
8 hr.	3·8	61·5	92·5	100
24 hr.	0·0	15·0	35·0	80

Table 2

Temperature	Node number 2	3	4	5	6	7	8	9
24° C.	4·5	13·7	27·5	32·0	41·0	50·0	54·5	54·5
15·5° C.	67	100	100	100	100	100	100	100

All produced male flowers at each node; but development of female flowers was strongly delayed at the higher temperature. Percentage femaleness, shown in Table 2, was determined as in the previous experiment.

Female plants of *Cannabis sativa* L. are known to produce male flowers under certain conditions[1]. My investigations indicate that environmental conditions affecting the sex of monoecious *Cucurbita* and *Mercurialis* affect female hemp similarly. Female plants can be induced to grow through a male phase before reaching their normal female expression.

When plants were grown in continuous light for 45 days at a day temperature of 21°–26° C. and a night temperature of 15·5°–21° C. and then transferred to 8-hr. photoperiods at the same temperature, male flowers were produced at the lowest flowering node (node 7 or 8) of the female plants.

These results are particularly interesting as they indicate the possibility that growth of female as well as monoecious plants through a male phase before they produce female flowers is a general phenomenon. Female plants normally reach the female phase before flowers develop and usually produce no male flowers, but by artificially delaying the attainment of femaleness, male flowers are produced.

The work is supported by a grant from the Agricultural Research Council, and experimental facilities have been supplied by the Nuffield Foundation.

R. G. Thomas

Department of Botany,
University College,
Nuffield Botanical Research Garden,
Nuffield Lodge,
London, N.W.1.
April 12.

[1] Nitsch, J. P., Kurtz, E. B., Liverman, J. L., and Went, F. W., *Amer. J. Bot.*, **39**, 32 (1952).
[2] Jones, K. L., *Amer. J. Bot.*, **34**, 371 (1947).
[3] Borthwick, H. A., and Scully, N. J., *Bot. Gaz.*, **116**, 14 (1954).

Soil Transmission of Scottish Raspberry Leaf-Curl Disease

Leaf-curl is the most important virus disease occurring in the raspberry-growing districts of eastern Scotland, where it has been known for many years[1]. It seems to be caused by raspberry ringspot virus, which can be transmitted by mechanical inoculation from raspberry plants with leaf-curl disease to plants of *Chenopodium amaranticolor* Coste and Reyn. and several other species[2]. None of the many kinds of arthropods tested as possible vectors transmitted the virus[3], and it seemed likely that the virus might be soil-borne.

In May 1955, soil was collected from the site of a recent severe outbreak of raspberry leaf-curl, potted and kept in a heated, insect-free glasshouse. Raspberry root-cuttings of the variety Malling Jewel were planted in the pots and also in pots containing the steam-sterilized potting mixture (John Innes potting compost No. 2) usually used. No symptoms of virus infection were observed in 1955; but in April 1956 fifteen out of thirty-two plants growing in soil from the leaf-curl site showed symptoms of leaf-curl. Local lesions were produced in leaves of *Chenopodium amaranticolor* inoculated with sap extracted from the leaves of each raspberry plant with symptoms: the *C. amaranticolor* plants were not infected systemically. Plants grown from root cuttings in sterilized potting mixture showed no symptoms of leaf-curl, and sap from them produced no lesions in leaves of *C. amaranticolor*.

It is also interesting that soil collected from the site of this particular leaf-curl outbreak harbours at least two other viruses. A virus of the tobacco-necrosis type was isolated from the first roots that grew on Malling Jewel root-cuttings planted in soil from the leaf-curl site: three out of eight plants contained the virus. No virus was detected in the roots of control plants grown in sterilized potting mixture. The virus resembled the tobacco necrosis viruses in producing large, discrete, necrotic lesions in the inoculated leaves of French bean variety Prince, in infecting plants of many other species without becoming systemic and in having a thermal inactivation point of between 90° and 95° C. when heated for ten minutes. No previous record is known of a virus of this type in raspberry.

Cadman[2] showed that sugar-beet seedlings became infected with a virus of the ringspot type when grown in potted soil from this leaf-curl site. I have repeatedly confirmed this finding by inoculating sap from macerated seedlings on leaves of *C. amaranticolor*, which develops local lesions and systemic symptoms when infected with this virus. Most of the infected seedlings were symptomless, and the virus appeared to be present more often in the roots than in the shoots of seedlings and never in the shoot only. No virus was isolated from sugar beet grown in sterilized potting mixture. Cross-protection tests in *Nicotiana tabacum* var. White Burley and in *Petunia nana* failed to show any relationship of the virus from sugar beet to raspberry ringspot virus. Its properties will be described elsewhere. Soils which, like the one used in these experiments, harbour three distinct plant viruses may be exceptional; but it seems more likely that soil-borne viruses are much commoner than has hitherto been suspected.

Raspberry ringspot virus and the ringspot virus from sugar beet both seem to resemble viruses of the tobacco-ringspot group. Many viruses of this type have been described, and they cause numerous diseases of economic importance: none is known to be transmitted by an arthropod vector from plants not infected by another virus, although it has been suggested that tobacco ringspot virus is transmitted by the aphid *Myzus persicae* from *Gladiolus* plants also infected with yellow bean mosaic virus to healthy *Gladiolus*[3]. The results described above now suggest that viruses of the tobacco-ringspot type may commonly be transmitted through the soil.

B. D. Harrison

Scottish Horticultural Research Institute,
Invergowrie,
by Dundee.
June 6.

[1] Harris, R. V., Bryce, A. D., and Foister, C. E., Rep. E. Malling Res. Sta. for 1942, 48 (1943).
[2] Cadman, C. H., *J. Hort. Sci.*, **31**, 111 (1956).
[3] Smith, F. F., and Brierley, P., *Plant Dis. Rep.*, **39**, 35 (1955).

FORTHCOMING EVENTS

Monday, September 10—Saturday, September 15

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH (at Government Offices, Horseguards Avenue, London, S.W.1)—Third International Congress on "High Speed Photography".

Thursday, September 13

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. A. D. Hibberd: "Recent Developments in Titanium Oxide Pigments".

Thursday, September 13—Monday, September 17

TEXTILE INSTITUTE (in the Congress House, Zurich, Switzerland)—Conference on "Static Electricity in Textiles".

Friday, September 14—Saturday, September 15

BIOCHEMICAL SOCIETY (on Friday in the Department of Biochemistry and Pharmacology, University College, Dublin, and on Saturday in the Department of Biochemistry, Trinity College, Dublin), at 10.30 a.m.—Scientific Papers.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LABORATORY MANAGER (with recognized engineering apprenticeship, and corporate membership of a senior professional Institution or equivalent qualifications, and broad experience in electrical and mechanical engineering)—The Establishment Officer, Atomic Energy Research Establishment, Harwell, Didcot, Berks, quoting Ref. 550/2 (September 17).

MATHEMATICAL PHYSICIST (with an honours degree and research or industrial experience) IN THE RESEARCH LABORATORIES, Central Electricity Authority, Leatherhead, to carry out work with a mathematical background in applied physics, such as flame radiation, stress analysis, fluid flow or temperature turbulence, and to assist colleagues with the mathematical side of their work—Director of Establishments, Central Electricity Authority, Winsley Street, London, W.1, quoting N/644 (September 17).

CHEMIST (with a university degree, or equivalent qualification, in chemistry, preferably with training in physics or geology, willing to spend periods of up to six months in the field on soil surveys) IN THE DEPARTMENT OF RESEARCH AND SPECIALIST SERVICES, Federation of Rhodesia and Nyasaland Ministry of Agriculture, for duties including routine and research work on soil fertility and on all related agricultural products and problems—Public Service Attaché, Rhodesia House, 429 Strand, London, W.C.2 (September 22).

RESEARCH ASSISTANT (with an honours degree in biochemistry or pharmacology) to work on the biochemistry of hypoglycæmic sulphonamides—Department of Pharmacology and Therapeutics, Queen's College, Dundee (September 22).

SCIENTIFIC OFFICER (with a university degree in agriculture or agricultural botany and a sound knowledge of the principles of genetics and practical plant breeding, preferably with experience in the inspection and certification of seed potatoes) IN THE PLANT BREEDING DIVISION, Ministry of Agriculture, Loughall, Co. Armagh, for work on potato breeding—The Secretary, Civil Service Commission, Stormont, Belfast, N. Ireland (September 24).

SCIENTISTS (with good honours degrees in physics, engineering, mathematics or a relevant social science, with ability to handle data and preferably with experience in industry or operational research) FOR THE SCIENTIFIC DEPARTMENT, Field Investigation Group, London Headquarters, National Coal Board, to assist in teams studying production and human problems in the coal industry—National Coal Board, Staff Department, Hobart House, London, S.W.1, quoting SS/379 (September 24).

SENIOR LECTURER IN PATHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, September 24).

LECTURER IN BACTERIOLOGY—The Secretary of University Court, University, Glasgow (September 28).

ORGANIC CHEMIST or BIOCHEMIST (preferably with experience), to undertake syntheses of radioactive compounds for metabolic investigations at the Pollards Wood Research Station, Institute of Cancer Research, Royal Cancer Hospital, Chalfont St. Giles—The Secretary, Chester Beatty Research Institute, Fulham Road, London, S.W.3, quoting JAVB (September 28).

LECTURER or ASSISTANT LECTURER (with special interest in general, inorganic or physical chemistry) IN CHEMISTRY—The Registrar, The University, Sheffield (September 29).

ASSISTANT LECTURER IN ECONOMICS—The Registrar, University College of North Staffordshire, Keele, Staffs (September 30).

ENGINEER (with an honours degree in physics or electrical engineering, or equivalent qualification, and with experience in the design and construction of electronic apparatus) IN CHARGE OF NUCLEAR EQUIPMENT in the Department of Physics—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield Road, Cambridge (October 1).

PHYSICAL CHEMIST or BIOCHEMIST for investigations on nucleoproteins and other biological substances—The Secretary, Chester Beatty Research Institute, Fulham Road, London, S.W.3, quoting "J.A.V.B." (October 1).

SENIOR LECTURER or LECTURER IN PHYSICAL CHEMISTRY or INORGANIC CHEMISTRY at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 12).

LECTURER IN SOCIOLOGY and a LECTURER IN BOTANY at the University of Natal—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 15).

FELLOW (chemist, with experience in radiochemical techniques) IN THE DEPARTMENT OF RADIOCHEMISTRY, Research School of Physical Sciences, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 29).

LECTURER (with a university degree in electrical engineering, preferably with experience in heavy electrical engineering and prepared to teach in this branch) IN ELECTRICAL ENGINEERING at the University of the Witwatersrand, Johannesburg—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (October 31).

DIRECTOR OF THE UNIVERSITY OF LONDON COMPUTATIONAL UNIT—The Secretary to the Senate, University of London, Senate House, London, W.C.1 (November 15).

AGRONOMIST (with a good honours degree and preferably with some practical experience) at the Gambia Rice Farm, to undertake experiments in the cultivation of irrigated rice and of alternative crops suitable for dry season cultivation under irrigation—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/12/08.

ASSISTANT EXPERIMENTAL OFFICER (with a knowledge of biochemistry or chemistry) to assist in metabolism studies in the Director's laboratory—The Secretary, Rowett Research Institute, Bucksburn, Aberdeenshire.

ASSISTANT REGIONAL TRIALS OFFICER (graduate in agriculture or botany, preferably with practical experience in crop production) at Norfolk Agricultural Station, Sprowston—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

CHEMIST (with at least five years research experience in some field of polymer chemistry), to lead a team to carry out a basic study of the mechanism, kinetics and properties of linear type polymers—The Director, Inveresk Gate, Musselburgh, Midlothian, Scotland.

CHIEF ENGINEER (with a degree in civil engineering and at least five years experience in high level administration in the engineering field) IN THE DEPARTMENT OF HOUSING, Jamaica, to take charge of engineering section covering subdivision layouts and development, construction of houses, and workshop producing prefabricated components for houses—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.160/32/02/T.

GEOLOGISTS (2) (with first- or good second-class honours degree in geology, preferably with a background of mathematics and physics) IN THE GEOLOGICAL SURVEY DEPARTMENT, for water supply work and general geological survey—The Director of Geological Survey, P.O. Lobatsi, Bechuanaland Protectorate.

GRADUATE (with a good honours degree in economics, preferably with statistics as a subsidiary subject, with an interest in manufacturing industries and aptitude for mathematical methods) in a department concerned with operational research and the relations and interactions of technology and economics, for duties primarily in problems concerning costs but also to participate in the other work of the department—Dr. D. W. Hill, Director of Research, The British Cotton Industry Research Association, Shirley Institute, Manchester 20.

GRADUATE IN ZOOLOGY or PHYSIOLOGY, to work on the breeding and feeding of small laboratory animals, mainly mice—The Director, Laboratory Animals Bureau, M.R.C. Laboratories, Holly Hill, Hampstead, London, N.W.3.

MATHEMATICS GRADUATE (with good knowledge of higher mathematics) for programming work for an electronic computer—The City Treasurer, City Hall, Norwich.

PHYSICIST or ELECTRICAL ENGINEER, Scientific Officer grade (with a first- or second-class honours degree in physics or electrical engineering, or equivalent qualification, and preferably with some electronics experience), with the Ministry of Supply, R.A.E., Aberporth, for research and development on electronic instrumentation—The Ministry of Labour and National Service, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.338/6A.

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (honours graduate, or equivalent, in chemistry or biochemistry), for research work on amino-acids in animal nutrition—The Secretary, Rowett Research Institute, Bucksburn, Aberdeenshire.

Editorial and Publishing Offices of "NATURE"

MACMILLAN & CO., LTD.,

ST. MARTIN'S STREET, LONDON, W.C.2.

Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., Crown House, 143–147 Regent Street, London, W.1

Telephone Number: Regent 3891

All rights reserved. Registered as a newspaper at the General Post Office